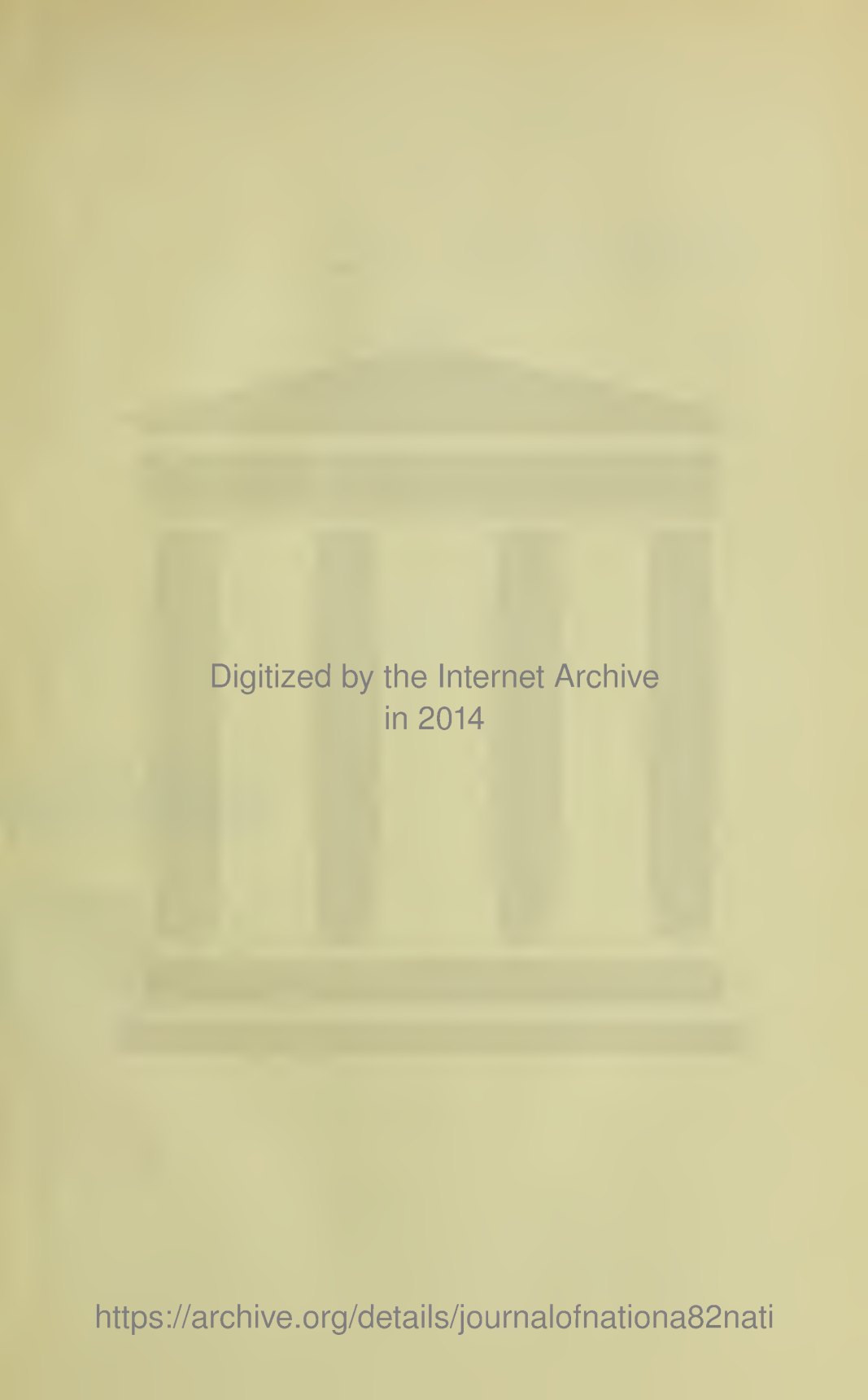

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MALARIA CONTROL PROGRESS AND PROBLEMS

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Reports of progress in the malaria control program being conducted jointly by the Communicable Disease Center of the Public Health Service and the respective State health departments have been made by Hollis (1946), Link (1947), and Tetzlaff (1948). These reports have shown, first, a gradual expansion of malaria control operations until a substantial coverage of the entire area in which malaria has been a problem in the United States was achieved and, second, the maintenance of this coverage for a sufficient period during which the continued absence of malaria deaths has made it logical for operations to be transferred to other areas having a malaria problem but not previously eligible for the control program. In the expansion and adjustment of areas approved for malaria control operations under this program, every effort has been made to secure the best possible epidemiological guidance so that Federal funds would be expended for control activities only in the most critical areas, and that the goal of malaria eradication from continental United States might be attained in the shortest possible period of time. The original criterion of an average annual death rate of 10 or more per 100,000 population during the period 1938–42, which was required for a county to be preapproved for malaria control operations with Federal funds, was reduced early in the program to an average annual death rate of 5 per 100,000 population. This more liberal requirement was made possible by substantial local participation in the cost of the program. With local funds almost equal to Federal funds, a considerably greater area could be given protection. (Fig. 1 and Table 1)

Since malaria mortality appears to have been virtually eliminated in certain of the counties presently approved for operations, it is apparent that further adjustments in the criteria are necessary. It appears that logical adjustments may be made by limiting approval to: (1) counties with an average annual death rate of 5 or more per 100,000 population during the period 1938–42, provided that those counties had an average annual death rate of 1 or more per 100,000 population during the period 1943–46; (2) counties which had an average annual death rate of 4 or more per 100,000 population during the period 1943–46; and (3) rural homes within one mile of a malaria case for which there has been laboratory confirmation by the State Health Department. (Figure 2 and Figure 3) The ultimate achievement of the goal of malaria eradication depends on a prompt adjustment of the areas of operation to conform to the area of disease prevalence, and it is hoped that with a further reduction of malaria morbidity and mortality increasing reliance may be placed on direct epidemiological guidance for the program.

The malaria control program has continued to consist almost exclusively of the use of DDT as a residual spray in homes requiring protection. Routine entomolog-

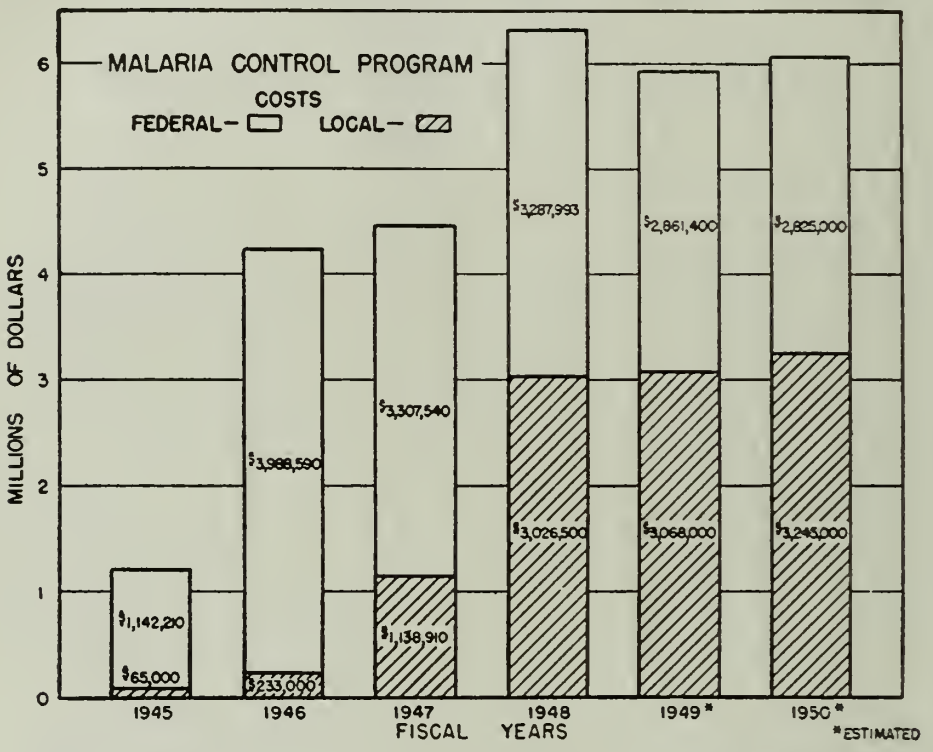

FIGURE 1. Participation by State and Local Sources in Cost of Joint Public Health Service-State Health Department Malaria Control Program.

TABLE 1

Summary of residual house-spraying operations

FISCAL YEAR	COUNTIES	HOUSE SPRAY APPLICATIONS	POUNDS DDT	POUNDS DDT PER HOUSE	MAN-HRS PER HOUSE
1945	111	264,482	103,957	0.39	1.75
1946	266	1,055,397	715,656	0.68	1.55
1947	297	1,236,841	964,449	0.78	1.28
1948	347	1,374,766	1,408,468	1.02	1.36
1949*	325	1,300,000	1,500,000	1.16	1.5

* Estimated.

ical inspections of the areas provided with such protection have demonstrated a substantial reduction in the number of houses found to be infested with mosquitoes, and satisfactory control of those mosquitoes likely to be malaria vectors in those areas. (Table 2)

Despite these achievements, however, there have been developments which, al-

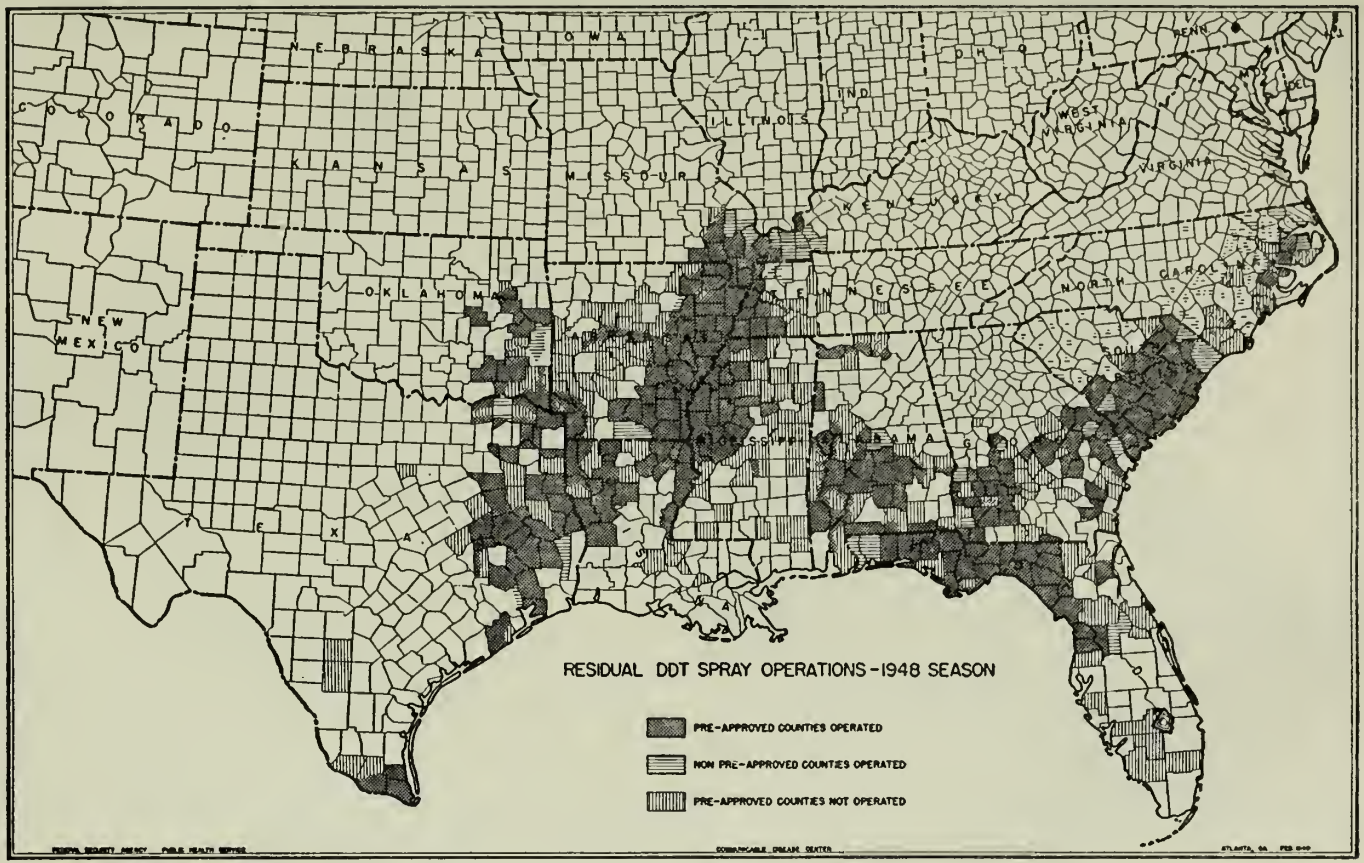

FIGURE 2. Extent of Residual DDT Spray Operations during 1948 Season.

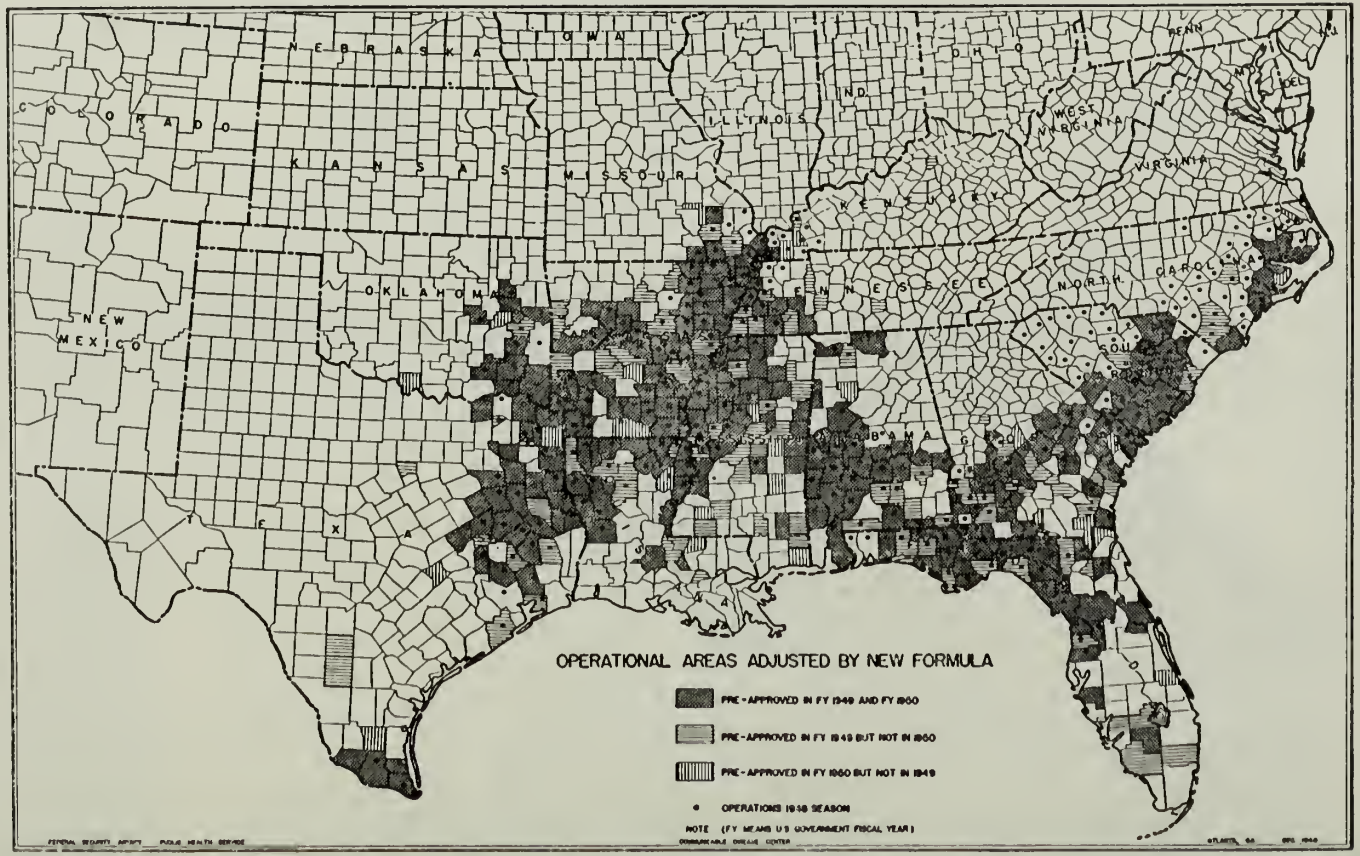

FIGURE 3. Adjustments Being Made in Areas Approved for Malaria Control Operations to Conform to Area of Disease Prevalence.

though not concerned in the control of malaria, may nevertheless have an important effect on the success of the program. During the past malaria control season, numerous reports were received from the operational areas to the effect that the concurrent fly control resulting from the residual DDT spraying program had not been as satisfactory as in previous years. Investigations which have been made thus far by various agencies to determine the extent to which a resistance to DDT may be developed in flies have indicated that, in areas where fly populations have been exposed to DDT, subsequent generations of the flies are likely to be more resistant (Fay and Buckner 1948). This phenomenon may in some measure be responsible for the apparent reduction in the effectiveness of fly control which has been reported. Many factors other than the development of DDT resistant strains of flies undoubtedly should share the responsibility for any apparent lack of fly control in areas where DDT residual spraying was carried out for malaria control purposes. Most

TABLE 2

Prevalence of A. quadrimaculatus in houses

	SPRAYED AREAS				UNSPRAYED AREAS		
	1945†	1946‡	1947‡	1948§	1946	1947	1948
Number of houses inspected*	14,129	21,951	9,083	7,479	1,639	1,170	1,021
Number of houses with *A. quadrimaculatus*	390	220	191	206	208	328	170
Per cent of houses with *A. quadrimaculatus*	2.8	1.0	2.1	2.8	12.7	28.0	16.7

* 0 to 5+ months after spraying.
† 100 mg. of DDT per sq. ft.
‡ 200 mg. of DDT per sq. ft.
§ 200 mg. of DDT per sq. ft., single application per season.

important are those pertaining to general sanitation, the unsatisfactory handling and disposal of garbage and refuse being of particular importance in this connection. Also, unsatisfactory DDT concentrates have been a factor in creating doubt of the effectiveness of the DDT being used in the minds of the men working with the insecticide, and of the people whose homes are being treated. On occasion discolored DDT concentrates, or concentrates which do not emulsify properly have been furnished by commercial producers. Under the circumstances, it may be necessary to revert to the original practice of making separate purchases of the constituent chemicals, namely DDT, emulsifier and solvent, and mixing the concentrates at various strategic points throughout the operational area. It is felt that this change alone will do much to eliminate the doubts which have arisen concerning the effectiveness of the program.

Also, it is being urged that emphasis again be placed on reminding the public that the purpose of the DDT residual spray program is malaria control, and where there is a demand for concurrent fly control, it be emphasized that this objective may be achieved only by the adoption of adequate sanitation practices.

Fly control will continue to be of importance in areas where the DDT residual spray malaria control program is being conducted, since that program is still very much in need of adequate local participation. Local participation has steadily increased, and it is felt that the extent of such participation is a measure of the local interest in the program and of the extent to which such activities will be incorporated as a part of the permanent public health program in the area. For this reason, consideration will continue to be given to the problem of fly control. The residual spraying program at present generally calls for a single seasonal application of DDT at a rate of approximately 200 mg. per square-foot of wall and ceiling surfaces in homes and on the back and undersides of furniture, on front and back porch and in privies. It is being recommended, however, that, if fly control is a particular problem in an area, consideration be given to the residual spray treatment of the remaining structures on the premises, such as the barn, stable, chicken coop, hog pen, etc. The Technical Development Division of the Communicable Disease Center has developed for this purpose a modified residual spray formula which incorporates the use of a sticking agent that makes the residual spray effective for much longer periods of time than emulsions which are recommended for use inside the homes.

Although the malaria control program of the Public Health Service still seems to be achieving adequate control of the vector of malaria by the use of DDT in residual spray treatment of homes, investigations are also being made into the suitability of other insecticides and different formulations. A number of these other insecticides could be used in the malaria control program whenever it is demonstrated that the use of DDT is no longer advisable or that the use of a substitute insecticide is indicated. DDT as it is now being used still appears to be the most effective and the most economical. With the modifications in the residual spray program being recommended for areas where fly control is a particular problem, it is believed that DDT will continue to prove effective in the malaria control program.

It is expected that continued progress will be made toward the goal of malaria eradication in continental United States. This progress will be due largely to improved insecticides and advances in malaria control being developed by adequate research. It will be implemented by the malaria control program being conducted jointly by the Public Health Service and the State health departments, and it should result in achieving the goal of malaria eradication in the five-year period which began in 1947 and which was originally set as the time required for this objective.

REFERENCES

Fay, R. W., and Buckner, Annette J. 1948. The Presence of DDT-Resistant Flies in the Field. Presented at Dec. 7, 1948 meeting of Nat. Mal. Soc.

Hollis, Mark D. 1946. Postwar Malaria Control in Continental U. S. J. Nat. Mal. Soc. **5:** 95–98 (Je. 1946).

Link, Vernon B. 1947. A Preliminary Report on Malaria Control by DDT Residual Spray. J. Nat. Mal. Soc. **6:** 124–130 (Je. 1947).

Tetzlaff, Frank. 1948. Operation of the Public Health Service Malaria Control Program. Pub. Health Repts. **63:** 557–563 (Apr. 30, 1948).

THE IDENTIFICATION AND DISTRIBUTION OF CHINESE ANOPHELINE MOSQUITOES[1]

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(Received for publication 9 March 1949)

Considerable additional information has accumulated about the distribution and habits of the anopheline mosquitoes of China since the publication of Feng's review of the records on these mosquitoes (1938). The total number of species known to occur in the area has increased from 26 to 38, largely through increased knowledge of the fauna of southern China (particularly Yunnan and Hainan Is.), and through the inclusion of the Taiwan fauna. A new appraisal of the total Chinese fauna would thus seem in order.

Most of the southern Chinese species are included in the key published by Christophers (1933). Some of these southern species, however, were not included in the key for northern Asian forms prepared by Russell, Rozeboom and Stone (1943). Consequently no single key exists which covers the whole Chinese fauna as presently known. The key included in the present paper is based on these previously published keys, but all of the characters have been tested with material of the various species collected in China.

Kowledge of the distribution of the different anophelines in China is of course very incomplete, since intensive studies have been limited to the coastal areas and to South China. The northwestern regions have been especially neglected, since malaria is not an important problem there, It seems unlikely that many forms new for the country will be found in that area.

Key to adult females

1.	Wing without pale markings	2
	Wing with pale markings	5
2.	Wing uniformly dark	3
	Wing with the scales at forks and at base of 2nd and 3rd veins clumped to form darker spots against the general grey background of the wing	4
3.	Head scales very narrow, rodlike	*aitkenii aitkenii* *aitkenii bengalensis*
	Head scales of ordinary type	*sintonoides*
4.	Mesonotum unicolorous; wing apex without a coppery spot in the fringe	*sacharovi*
	Mesonotum darker at sides; apex of wing with a coppery spot in the fringe	*maculipennis*

[1] The studies reported in this paper were carried out under the auspices of the National Institute of Health of China in cooperation with the International Health Division of The Rockefeller Foundation. I am indebted to Dr. S. C. Hsu, head of the Malaria Division of the Institute, and to Dr. M. C. Balfour and Dr. R. B. Watson of The Rockefeller Foundation for help and encouragement. I want to express my special thanks to Dr. Marston Bates of The Rockefeller Foundation for help in the preparation of the manuscript.

TABLE I

*Distribution of Chinese anophelines by provinces**

Species	PROVINCES																
	Anhwei	Chekiang	Fukien	Hainan Is	Hopei	Hunan	Kiangsi	Kwangsi	Kwantung	Keichow	N.E. 9 Provinces	Shantung	Sikang	Szechuan	Taiwan	Tibet	Yunnan
1. *aconitus*				+													+
2. *aitkenii aitkenii*		+				+	+										+
3. *aitkenii bengalensis*		+					+		+						+		+
4. *annandalei interruptus*																	+
5. *annularis*			+	+				+							+		+
6. *barbirostris*				+					+					+			+
7. *barbumbrosus*															+		
8. *culicifacies*																	+
9. *fluviatilis*			+						+					+	+		+
10. *gigas baileyi*										+				+	+	+	+
11. *gigas simlensis*										+						+	
12. *hyrcanus nigerrimus*				+													+
13. *hyrcanus sinensis*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
14. *insulaeflorum*															+		
15. *jamesii*				+													+
16. *jeyporiensis candidiensis*		+	+	+				+	+						+		+
17. *karwari*								+	+								+
18. *kochi*				+				+	+								+
19. *koreicus*		+															
20. *kweiyangensis*										+							
21. *leucosphyrus*				+											+		+
22. *lindesayi japonicus*					+							+	+	+			
23. *lindesayi lindesayi*	+	+	+			+	+			+					+		+
24. *ludlowii*				+											+		
25. *maculatus*			+	+		+	+	+	+	+				+	+		+
26. *maculipennis*											+						
27. *minimus*		+	+	+		+	+	+	+	+			+	+	+		+
28. *pattoni*					+							+	+	+			
29. *philippinensis*				+													+
30. *sacharovi*																	
31. *sineroides*		+															
32. *sintonoides*				+													
33. *splendidus*			+	+				+	+						+		+
34. *stephensi*																	+
35. *subpictus indefinitus*				+											+		
36. *subpictus subpictus*									+								+
37. *tessellatus*				+					+						+		+
38. *vagus*				+				+	+								+

* There are no records for the provinces of Chahar, Jehol, Kansu, Mongolia, Ningsia, Shensi, Suiyüan and Tsinghai. Only one species, *A. hyrcanus sinensis*, has been reported in the provinces of Honan, Hupeh, Kiangsu and Shansi. For the province of Sinkiang only *A. sacharovi* has been reported.

5. Wing with less than four dark areas on costa, including both the costa and vein 1 6
Wing with at least four dark areas on costa 16
6. Hind femur with outstanding tuft of black and white scales at its distal end ..... *annandalei interruptus*
Hind femur not so 7
7. Hind femur with a broad white band at middle 8
Hind femur without such band 9
8. Basal third of hind femur in ventral aspect pale ..... *lindesayi lindesayi*
Basal $\frac{1}{6}$, or less, of hind femur pale ventrally ..... *lindesayi japonicus*
9. Inner quarter of costa mostly pale 10
Inner quarter mainly dark, though there may be scattered pale scales 11
10. Fringe dark at vein 3 and at all veins below 3, except for the usual pale spot between 5.2 and 6 ..... *gigas baileyi*
Fringe pale at vein 3 and often at other vein as well ..... *gigas simlensis*
11. Palpi with pale distinct rings 12
Palpi without pale distinct rings 14
12. Costa with pale humeral spot; vein 6 with three dark spots ..... *kweiyangensis*
Costa without pale humeral spot; vein 6 with two usual dark spots 13
13. Fourth hind tarsal segment with pale basal band ..... *hyrcanus nigerrimus*
Fourth hind tarsal segment without pale basal band ..... *hyrcanus sinensis*
14. Basal third of costa with scattered white scales 15
Base of costa with two small white spots ..... *koreicus*
15. Wing apex mostly dark except on tip of vein 3; venter of abdomen with pale scales ..... *barbirostris*
Wing apex mostly pale except on tip of vein 2.2; venter of abdomen without pale scales ..... *barbumbrosus*
16. Apex of hind tarsus not white 17
Apex of hind tarsus white 28
17. Vein 6 with more than three dark spots ..... *tessellatus* (in part)
Vein 6 with three or less dark spots 18
18. Fore tarsi with broad pale bands 19
Fore tarsi unbanded, or with only very narrow, pale bands 23
19. Femora and tibia speckled 20
Femora and tibia not speckled 21
20. Palpi with a single broad, apical, pale band ..... *ludlowii*
Palpi with two broad, apical, pale bands ..... *stephensi*
21. Wing subapical dark costal spot as long as, or longer than, pale spot on either side ..... *subpictus indefinitus*
Wing subapical dark costal spot shorter than pale spot on either side 22
22. Intervening dark area of female palpi equal to, or nearly equal to, the pale apical band ..... *subpictus subpictus*
Intervening dark palpal area half, or less than half, the length of the pale apical band ..... *vagus*
23. Costa with four pale spots, the first two small and situated at the extreme base of the wing; no sectoral spot ..... *sineroides*
Costa with more than four pale spots; if with only four pale spots, both the first and second are not situated at extreme base of wing; sectoral spot present 24
24. Fringe spot present at vein six 25
Without fringe spot at vein six 26

25. Palpi with two unequal, pale bands, the apical one broader than the subapical; basal area of costa with two pale interruptions ..... *jeyporiensis candidiensis*
Palpi with two equally broad, pale bands; basal area of costa either without or with a single pale interruption ..... *aconitus*
26. Palpi with the two apical pale bands as broad as, or broader than, the intervening dark area ..... *minimus*
Palpi with subapical pale band narrow, and the intervening dark area much broader 27
27. Vein 3 usually extensively pale; fringe spots present on all veins but 6 ..... *fluviatilis*
Vein 3 all dark; fringe spots on one or two veins only ..... *culicifacies*
28. Tarsi with not more than one terminal segment, white, or without white markings entirely 29
Tarsi with more than two terminal segments continuously white 34
29. Femora and tibia not speckled 30
Femora and tibia speckled 31
30. Palpi with two broad apical white bands and a single narrow basal white band ..... *pattoni*
Palpi with two broad apical white bands and two narrow basal white bands ..... *karwari*
31. Vein 6 with more than three dark spots 32
Vein 6 with three or fewer dark spots 33
32. Hind (tibio-tarsal joint) with broad, conspicuous white band ... *leucosphyrus*
Hind (tibio-tarsal joint) without such a band ..... *tessellatus* (in part)
33. Venter of abdomen with row of conspicuous, black scale-tuft, (these being clearly visible to the naked eye, lateral view) .... *kochi*
Abdomen not so ..... *maculatus*
34. Femora and tibia not speckled 35
Femora and tibia speckled 36
35. Stem and lower branch of vein 5 mainly dark or at least with a dark spot at the origin of the branch ..... *annularis*
Stem and lower branch of vein 5 mostly white, only with a dark spot at each end ..... *philippinensis*
36. Palpi with two broad apical pale bands, one narrow band, and conspicuous speckling ..... *splendidus*
Palpi with a single broad, apical, pale band, two narrow bands, and no speckling ..... *jamesii*

Key to full-grown larvae[2]

1. *ic* more or less approximated 2
ic well separated 15
2. Antennal hair simple 3
Antennal hair branched 4
3. *oc* simple ..... *sintonoides*
oc branched ..... *annandalei interruptus*
4. *oc* simple, bifid, or with at most a few *short* branches 5
oc with many *long* branches 10
5. *ic* split at about their middle into 2–5 branches, bases not close together 6
ic simple; bases nearly touching 7

[2] *ic* denotes inner clypeal hair; *oc* denotes outer clypeal hair. The larvae of *koreicus* and *sineroides* are not available for the preparation of the key.

6. *ic* split into 2 parts ..... *aitkenii aitkenii*
   ic split into 3–5 parts ..... *aitkenii bengalensis*
7. Palmate hair on abdominal segment 1 well developed ..... *insulaeflorum*
   Palmate hair on abdominal segment 1 not developed, but hair-like 8
8. Palmate hairs present on abdominal segments 2–7; thoracic palmate hair well developed ..... *lindesayi lindesayi*
   lindesayi japonicus
   Palmate hairs present on abdominal segments 3–7; thoracic palmate hair not differentiated 9
9. Long lateral hair on abdominal segment 5 simple ..... *gigas baileyi*
   This hair split into 2–3 branches ..... *gigas simlensis*
10. Prothoracic hair 1 (inner shoulder hair) simple, or split into two or three branches at tip 11
    Prothoracic hair 1 branched near base 14
11. Antennal hair very long, strongly plumose, and situated at about the middle of antenna 12
    Antennal hair of moderate length, weakly branched, and situated before the middle of antenna 13
12. *oc* with about 20 branches, not forming a broomlike tuft ..... *kweiyangensis*
    oc with about 30–50 branches, forming a strong, broomlike tuft ..... *hyrcanus sinensis*
    hyrcanus nigerrimus
13. Hair no. 2 (antepalmate) of abdominal segments 4–5 with less than 9 branches ..... *maculipennis*
    This hair with more than 9 branches ..... *sacharovi*
14. *oc* with 12–20 branches, not forming a broomlike tuft ..... *barbumbrosus*
    oc with 30–50 branches, forming a strong, broomlike tuft ..... *barbirostris*
15. Anterior tergal plates on abdominal segments 3–7 very large, with a convex posterior border, and enclosing the small, oval, posterior tergal plate 16
    These plates not exceptionally large, with a concave posterior border, and not enclosing the small, oval, posterior tergal plate 17
16. All clypeal hairs, including the posterior one, simple ..... *minimus*
    fluviatilis
    ic and *oc* with short, scattered branches; posterior clypeal hair with 2–3 branches ..... *aconitus*
17. *ic* and *oc* simple, or with short, inconspicuous lateral fraying 18
    ic and *oc* with lateral branches or conspicuous fraying 27
18. Abdominal segment 1 with distinct palmate hair 19
    Abdominal segment 1 without distinct palmate hair 24
19. All thoracic pleural hairs simple ..... *kochi*
    Some of the thoracic pleural hairs branched 20
20. Thoracic palmate hair distinct ..... *culicifacies*
    Thoracic palmate hair not differentiated 21
21. *ic* and *oc* with very small branches ..... *pattoni*
    ic and *oc* simple 22
22. *oc* not more than $\frac{1}{3}$ as long as *ic*; posterior clypeal hair internal to, and close to, the *ic* ..... *vagus*
    oc about $\frac{1}{2}$ or more as long as *ic*; posterior clypeal hair external to, and quite far from, the *ic* 23
23. Antennal hair arising nearer base than apex of antenna ..... *subpictus subpictus*
    subpictus indefinitus
    Antennal hair arising about the middle of antenna ..... *ludlowii*

24. Abdominal segment 2 with distinct palmate hair..... 25
Abdominal segment 2 without distinct palmate hair..... 26
25. *ic* and *oc* finely frayed..... *maculatus* (in part)
ic and *oc* simple..... *stephensi*
26. Prothoracic hair 1 with only 2–4 branches and arising from an inconspicuous root..... *tessellatus*
Prothoracic hair 1 with numerous branches, root large and dark brown..... *leucosphyrus*
27. *oc* with long branches, almost as long as the hair itself..... 28
oc with short lateral branches, at most $\frac{1}{6}$ the length of the hair... 30
28. Inner sutural (or occipital) hair simple, or bifid near tip..... 29
This hair split near base into 2–8 branches..... *philippinensis*
29. Abdominal segment 1 with well-developed palmate hair..... *annularis*
Abdominal segment 1 without differentiated palmate hair.... *jamesii*
30. Palmate hairs on metathorax and abdominal segment 1 well developed; anterior tergal plates rather large..... *jeyporiensis candidiensis*
Palmate hairs on metathorax and abdominal segment 1 not differentiated; anterior tergal plates much smaller..... 31
31. *oc* split into two, and with 3–7 short lateral branches; inner sutural hair split into two or several parts..... *splendidus*
oc with a few fine lateral branches; inner sutural hair simple.. 32
32. Long lateral hair on abdominal segments 5–6 split near base into three to five branches..... *maculatus* (in part)
This hair with a central main stem and six to ten long branches arising along the length of the stem..... *karwari*

Notes on species

Distribution of anophelines by provinces is summarized in Table I, and further information is given in the following list. Where no authority is quoted for a particular record, it is taken from the review published by Feng (1938). The notes on larval habitats are based mostly on experience in southern China. In a few cases, where these seemed particularly important or interesting, comments on the habits of the adults and on the relation of the species to malaria have been added.

1. *A. aconitus* Dönitz
Hainan Is. (Takei, 1941); Yunnan (Sweet *et al.*, 1942)
Breeds in irrigation channels and rice fields.
This species is apparently not important as a malaria vector in China because of its scarcity; it was mostly found resting in cow sheds.
2. *A. aitkenii aitkenii* James
Chekiang; Hunan (Chang, 1939); Kiangsi; Yunnan.
Breeds in shaded cool pools.
3. *A. aitkenii bengalensis* Puri
Chekiang; Kiangsi; Kwangtung; Taiwan (Chow, 1948b); Yunnan.
Larval habitat same as that of the preceding species.
4. *A. annandalei interruptus* Puri
Yunnan (Sweet *et al.*, 1942)
Breeds in tree holes in jungle.
5. *A. annularis* van der Wulp

Fukien (Chow and Chang, 1943); Hainan (Takei, 1941); Kwangsi; Taiwan (Chow, 1948b); Yunnan.

Breeds in ponds, pools and rice fields.

This species bites both man and cattle; it is common, but most females leave the biting place at dawn, so that its abundance cannot be judged by the numbers of resting specimens. Var. *adiei* James and Liston, recorded by Yao and Ling (1937) in south China is considered by Christophers (1933) to be a seasonal form.

6. *A. barbirostris* van der Wulp

Hainan; Kwangtung; Szechuan (Meng, 1943); Yunnan.

Breeds in ponds, pools and rice fields.

7. *A. barbumbrosus* Strickland and Chowdhury

Taiwan (Chow, 1948b).

Breeds in shaded pools.

This species is rare.

8. *A. culicifacies* Giles

Yunnan.

Breeds in irrigation channels, rice fields and pools.

Too scarce to be important in China, although it is the chief malaria vector in Ceylon and certain parts of India. Gaschen (1934) found oöcysts in one specimen in Yunnan, but several hundred dissections that we made in the same province were negative (Sweet *et al.*, 1942).

9. *A. fluviatilis* James

Fukien (Chow and Chang. 1943); Kwangtung; Szechuan (Chow, unpublished); Taiwan (Chow, 1948b); Yunnan (Sweet *et al.*, 1942). Larval habitat same as that of *A. minimus*.

10. *A. gigas baileyi* Edwards

Kweichow; Szechuan; Tibet; Taiwan (Chow, 1948b); Yunnan.

Breeds in cool water pools, in mountainous regions.

11. *A. gigas simlensis* James

Kweichow; Tibet.

Larval habitat same as that of preceding species.

12. *A. hyrcanus nigerrimus* Giles

Hainan; Yunnan.

Breeds in rice fields, ditches and ponds.

13. *A. hyrcanus sinensis* Wiedemann

Generally distributed in China.

Breeds in both still and running water, but more commonly in rice fields.

The bionomics of this species in China has been discussed by Chow (1948a). It is the chief vector of malaria in the plain of central China, though its natural infection rate is low (0.1–0.3%).

14. *A. insulaeflorum* (Swellengrebel and S. de Graf)

Taiwan (Chow, 1948b).

Breeds in shaded cool pools.

15. *A. jamesii* Theobald
Hainan; Yunnan (Chang, 1940).
Breeds in river-bed pools.

16. *A. jeyporiensis candidiensis* Koidzumi
Chekiang; Fukien; Hainan; Kwangsi; Kwangtung; Taiwan (Chow, 1948b); Yunnan.
Breeds in seepage water and fallow rice fields.
This species is regarded as of secondary importance in malaria transmission in the hilly regions of south China.

17. *A. karwari* James
Kwangsi; Kwangtung; Yunnan.
Breeds in slowly running streams and pools.

18. *A. kochi* Dönitz
Hainan (Ho, 1938); Kwangsi; Kwangtung; Yunnan. Its presence in Taiwan is doubtful (Chow, 1948b).
Breeds in rice fields and pools.

19. *A. koreicus* Yamada and Watanabe
Chekiang.
Breeds in cool spring pools on mountains.

20. *A. kweiyangensis* Yao and Wu
Kweichow (Yao and Wu, 1944).
Breeds in ground pools.

21. *A. leucosphyrus* Dönitz
Hainan (Takei, 1941); Yunnan (Sweet *et al.*, 1942); Taiwan (Chow, 1948b)
Breeds in shaded pools in mountain stream beds.

22. *A. lindesayi japonicus* Yamada
Hopei; Shangtung; Sikang and Szechuan (Crook, 1939).
Breeds in pools or spring water on mountains.
Crook (1939) recorded in Sikang and Szechuan the presence of var. *pleccau* Koidzumi, which is considered to be a synonym of *A. lindesayi japonicus*.

23. *A. lindesayi lindesayi* Giles
Anhwei; Chekiang; Fukien; Hunan (Chang, 1939); Kiangsi; Kweichow; Taiwan (Chow, 1948b); Yunnan.
Larval habitat same as that of preceding species.
Feng (1938) writes that "it is not certain whether this (var. *japonicus*) is the only form present in China, or whether the type form or its other varieties also exist." I have examined the specimens collected from Taiwan and Yunnan and found these to be the type form.

24. *A. ludlowii* Theobald
Hainan (Takei, 1941); Taiwan (Chow, 1948b).
Breeds in stream bed pools with sandy bottoms and without vegetation, and also in grassy edges of streams, and occasionally in rice fields.
This species rests during the day in cowsheds, empty houses, ground holes, and occasionally in tree buttresses. It is prevalent during the months from December to April, reaching its peak in February. No plasmodial infection was

found in about three thousand dissections. The wing pattern of this species varies, so that it sometimes looks like that of *sundaicus*, i. e., two dark spots (instead of three) on vein 1 below median dark area on costa, and fringe without spot between vein 6 and 5.2.

25. *A. maculatus* Theobald
Fukien; Hainan (Ho, 1938); Hunan (Chang, 1939); Kiangsi; Kwangsi; Kwangtung; Kweichow; Szechuan (by Dr. C. Ho); Taiwan (Chow, 1948b); Yunnan.
Breeds in stream and river-bed pools with sandy or stony bottom.
Although this species has been found naturally infected in south China, it seems not to be important in malaria transmission, the chief vector being *minimus*. Ho (1938) reported his specimens from Hainan as of var. *hanabusai* Yamada. This is usually regarded as a synonym.

26. *A. maculipennis* Meigen
Heilungkiang (Northeastern nine provinces).
Breeds in ditches containing fresh water.
From the epidemiological point of view, this species has been regarded as responsible for malaria transmission in Heilungkiang (Feng, 1937).

27. *A. minimus* Theobald
Chekiang; Fukien; Hainan; Hunan (Chang, 1939); Kiangsi; Kwangsi; Kwangtung; Kweichow (by Dr. H. C. Kan); Sikang and Szechuan (Crook, 1939); Taiwan (Chow, 1948b); Yunnan.
Breeds in slowly running water, such as streams, irrigation channels, ditches, Occasionally recorded from ground pools and rice fields.
A. minimus has proved to be the most important malaria vector in south China. The bionomics of this species has been discussed in more detail by Chow (1948a).

28. *A. pattoni* Christophers
Hopei; Shangtung; Sikang (Crook, 1939); Szechuan.
Breeds in hilly streams and pools.
This species is considered, from the epidemiological point of view, to be an important malaria vector in the hilly regions of north China, though natural infection has not yet been found (Feng, 1937).

29. *A. philippinensis* Ludlow
Hainan (Ho, 1938); Yunnan.
Breeds in ponds, ditches, rice fields.

30. *A. sacharovi* Favr
Sinkiang.
Epidemiologically this species is considered to be a vector of malaria in Kashgar, Sinkiang (Feng, 1937).

31. *A. sineroides* Yamada
Chekiang.
Breeds in cool spring pools.

32. *A. sintonoides* Ho
Hainan (Ho, 1938).
Breeds in tree holes.

33. *A. splendidus* Koidzumi

Fukien; Hainan; Kwangsi; Kwangtung; Taiwan (Chow, 1948b); Yunnan.
Breeds in stream-bed pools, and ditches.

34. *A. stephensi* Liston
Yunnan (Williams, 1941; Sweet *et al.*, 1942).
This record is based on a single female hatched out from some larvae collected by Dr. K. C. Chen in 1940.

35. *A. subpictus indefinitus* Ludlow
Hainan (Ho, 1938); Taiwan (Chow, 1948b).
Breeds in pools in fallow rice fields, streams, ditches.

36. *A. subpictus subpictus* Grassi
Kwangtung; Yunnan (Sweet *et al.*, 1942)
Breeds in animal hoof marks.

37. *A. tessellatus* Theobald
Hainan; Kwangtung; Taiwan (Chow, 1948b); Yunnan (Chang, 1940).
Breeds in ditches, pools in fallow rice fields, and swamps.
The daytime resting places are cow sheds, ground holes, tree buttresses and grass.

38. *A. vagus* Dönitz
Hainan; Kwangsi; Kwangtung; Yunnan.
Breeds in animal hoof marks.

REFERENCES

Chang, T. L. 1939. Mosquitoes of Hunan Province with special reference to *Anopheles*. Chinese Med. J., **56:** 52–62.

1940. The anopheline mosquitoes of Yunnan. Chinese Med. J. **58:** 218–233.

Chow, C. Y. 1948a. The bionomics of two important malaria vectors in China. Proc. 4th Intern. Congr. Trop. Med. & Mal., Washington (in press).

1948b. The anopheline mosquitoes of Taiwan (Formosa), China (in manuscript).

Chow, M. T., and Chang, M. C. 1943. Known species of *Anopheles* from Fukien. New Agri. J., **3:** 112–134 (in Chinese, with English summary).

Christophers, S. R. 1933. The Fauna of British India. Diptera, Vol. IV. Family Culicidae. Tribe Anophelini. London: Taylor & Francis, xii + 371 pp., 53 figs., 3 pl.

Crook, R. L. 1939. Some notes on malaria in Szechuan. Chinese Med. J., **55:** 465–478.

Feng, L. C. 1937. The anopheline mosquitoes and the epidemiology of malaria in China. Chinese Med. J., **51:** 1005–1020.

1938. A critical review of literature regarding the records of mosquitoes in China. Peking Nat. History Bull., **12:** 169–181.

Gaschen, H. 1934. Recherches entomologiques dans la Province du Yunnan. Bull. Soc. Méd.-chir. Indochine, **12:** 873–892.

Ho, C. 1938. On a collection of anopheline mosquitoes from the Island of Hainan. Ann. Trop. Med. & Parasitol., **32:** 387–411.

Meng, C. H. 1943. Notes on the *Diptera* of medical importance in Chengtu. J. West China Border Res. Soc. (B), Chengtu, **14:** 99–101.

Russell, P. F., Rozeboom, L. E., and Stone, A. 1943. Keys to the anopheline mosquitoes of the world with notes on their identification, distribution, biology, and relation to malaria. Philadelphia: Acad. Nat. Sci., 152 pp., 10 figs.

Sweet, W. C., Feng, L. C., Chow, C. Y., and Hsau, S. C. 1942. Anophelines of Western Yunnan and their relation to malaria. J. Nat. Malaria Soc., **1:** 25–32.

TAKEI, H. 1941. A field investigation on the species of *Anopheles* in Hainan Island and their distribution. Kaigun Gunigakkai Zassi (J. Nav. Med. Assoc.) [In Japanese], **30:** 414–437.

WILLIAMS, JR. L. L., 1941. Malaria on the China Burma Highway. Amer. J. Trop. Med., **21:** 1–11.

YAO, Y. T., AND LING, L. C. 1937. Study of mosquito fauna in southwestern China especially in the provinces of Kweichow and Yunnan. Japanese J. Exp. Med., **15:** 121–136.

YAO, Y. T., AND WU, C. C. 1944. A new anopheline mosquito, *Anopheles kweiyangensis*, sp. n., from Kweiyang, Kweichow. [In Chinese] China Med. J. **30:** (1).

NMS MEETS MEMPHIS, NOV. 6–9

The thirty-second annual meeting of the National Malaria Society will be held in Memphis, Tenn., Nov. 6–9, 1949. The first general meeting of the Society was held in Memphis 32 years ago. Since then, the Society has met twice in Memphis, in 1927 and in 1939. The Society will meet conjointly with the American Society of Tropical Medicine and the American Academy of Tropical Medicine.

Registration will begin on the afternoon of Nov. 6. Scientific sessions are scheduled for the 7th, 8th and 9th. Headquarters will be at the Hotel Peabody. Hotel reservation blanks and applications for a place on the program and for exhibit space will be mailed to members from the Office of the Secretary.

THE ASCORBIC ACID CONTENT OF THE ADRENAL GLANDS OF CHICKS INFECTED WITH *PLASMODIUM GALLINACEUM*

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Numerous references to a relationship between malarias in primates and vitamin C deficiency have appeared in the literature. Reports by Lotze (1938), Gerdjikoff (1939), Krishnan (1940), Mohr (1943), Reniger-Arshiva (1944), Sorce and Mutolo (1946) and Wozonig (1946) indicated that humans infected with *Plasmodium vivax* and/or *P. falciparum* became depleted of ascorbic acid. McKee and Geiman (1946) and McKee *et al.* (1947) reported similar findings in monkeys infected with *P. knowlesi*. The depletion of this vitamin may be due partially to the metabolic activities of the malaria parasite itself, since ascorbic acid is required for *in vitro* cultivation of *P. knowlesi* (Ball *et al.*, 1945).

We were interested in observing the effect of a malarial infection on the adrenal ascorbic acid concentration of an animal such as the chick which does not require this vitamin in the diet (Biester and Schwarte, 1948). Since chick adrenals contain relatively high concentrations of this vitamin, a systematic examination of the adrenal ascorbic acid of chicks infected with *P. gallinaceum* was conducted and the results are reported here.

METHODS

All chicks were infected with an inoculum of 1000 chick erythrocytes parasitized with *P. gallinaceum*. The methods used in infecting the chicks and removing and weighing the adrenals are described elsewhere (Nadel *et al.*, 1949). Pairs of adrenals from 3 to 5 chicks of the same age and stage of parasitemia were pooled and analyzed for ascorbic acid content by the titrimetric method of Bessey (Hawk *et al.*, 1947). Non-infected chicks from the same lot served as controls. The glands, frozen in solid carbon dioxide, were homogenized with 50 ml. of a cold 8 per cent acetic-3 per cent metaphosphoric acid mixture for 1 minute in a semi-micro Waring Blendor. Aliquots of the homogenates were titrated in duplicate or triplicate. Losses in ascorbic acid content were kept to a minimum by performing the assays within 15 minutes after the chicks were sacrificed. All adrenal samples were kept at $-70°$ C. during the interval between dissection and assay.

EXPERIMENTAL

The mean ascorbic acid concentrations in the adrenals of 117 infected chicks between 7 and 17 days after inoculation of parasites (during patency[2]) and 74 non-

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[2] Arbitrarily, the period in the infection during which parasite density $\geqq 5$ per 10^4 erythrocytes, the approximate minimum density detectable in thin blood smears by our techniques of examination.

infected controls are shown in table 1. There is no significant difference in the adrenal ascorbic acid concentrations (milligrams ascorbic acid per gram of fresh adrenal tissue) of infected and non-infected chicks even though the infected chicks were subjected for periods up to 17 days to malaria, a stress agent usually resulting in death in over 95 per cent of the individuals. Likewise, during the prepatent period (days 0 through 6 after inoculation of parasites) no significant differences in adrenal ascorbic

TABLE 1

Ascorbic acid concentrations in the adrenal glands of chicks between 7 and 17 days after infection with 10^3 chick erythrocytes parasitized with Plasmodium gallinaceum *compared with those of non-infected controls*

	NO. CHICKS	ADRENAL WT./PAIR	ADRENAL ASCORBIC ACID		
			No. assays*	mg./gm.†	mg./pair
		mg.			
Infected chicks	117	23.1 ± 1.58	28	1.58 ± 0.05	0.037 ± 0.003
Non-infected controls	74	17.6 ± 0.99	16	1.56 ± 0.08	0.027 ± 0.002
Significance		$P < 0.01$		$P > 0.8$	$P < 0.01$

* Pairs of adrenals from 2 to 5 chicks were pooled for each assay.

† Range 1.06–2.31.

TABLE 2

The ascorbic acid content of adrenal glands of chicks infected with Plasmodium gallinaceum *during the prepatent period compared with uninfected controls*

TIME AFTER INOCULATION	MEAN ADRENAL ASCORBIC ACID CONTENT* (MG./GM. OF FRESH TISSUE)	
	Infected chicks	Control chicks
hours		
1	1.35	1.38
20	1.30	1.35
44	1.43	1.15
68	1.25	1.63
92	1.15	1.37
116	1.37	1.43

* Mean values are based upon duplicate determinations of two samples consisting of 5 pairs of adrenals per sample.

acid concentrations between infected chicks and non-infected controls could be found (table 2).

To determine the effect of the administration of ascorbic acid on the course of parasitemia, infected chicks of the same lot, age and weight were divided into test and control groups. The test group was injected intraperitoneally with 0.5 ml. of a 2 per cent ascorbic acid solution in distilled water once daily (10 mg. per day) beginning 4 hours prior to inoculation with parasites and continuing until death from malaria. Daily parasite counts were made from the onset of patency until death. As may be seen in figure 1 and table 3, this treatment with ascorbic acid did not influence in any way the course of the infection. Ascorbic acid determinations were not performed on the adrenals of chicks injected with this vitamin.

In order to ascertain the effect of deprivation of dietary ascorbic acid upon the ascorbic acid concentration in the chick adrenal, a series of 24-day-old uninfected chicks of the same lot was divided into Groups A, B, and C. Group A was sacrificed immediately and the adrenal ascorbic acid assays were performed. Group B was permitted access to feed (starting mash) and water *ad libitum*. Group C was de-

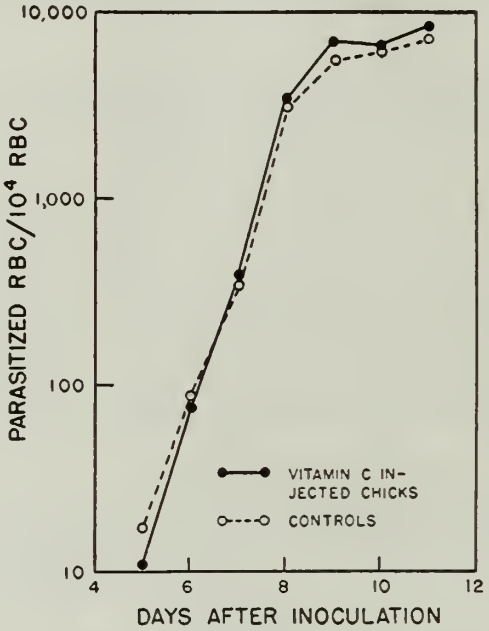

FIG. 1. Course of a *P. gallinaceum* infection in chicks untreated and treated with ascorbic acid.

TABLE 3

Mean days of first parasitemia and of death from infection with Plasmodium gallinaceum *of chicks untreated or treated with ascorbic acid*

	NO. CHICKS	MEAN PREPATENT PERIOD	MEAN SURVIVAL AFTER INOCULATION
		days	*days*
Treated	10	5.0	11.0
Untreated	10	5.6	10.6*

* Two chicks which survived the acute parasitemia but died of exoerythrocytic forms are not included.

prived of both food and water for 5 days. At the end of this period the chicks in Groups B and C were sacrificed and the adrenal ascorbic acid values were obtained (table 4). Any differences among the groups are of no statistical significance.

DISCUSSION

From the literature reports previously cited, varying degrees of depletion of vitamin C occur in primates infected with malaria. McKee and Geiman (1946) were

able to reduce the rate of multiplication of *P. knowlesi* in the monkey by withholding dietary ascorbic acid. Subsequent administration of this vitamin to the vitamin C deficient monkeys was followed by normal parasite development. McKee *et al.* (1947) also found sharp decreases in adrenal ascorbic acid in monkeys during the course of the infection with *P. knowlesi*. By analogy one might expect lowered ascorbic acid values in chick adrenals at some time during the course of *P. gallinaceum* infections. No such reduction, however, was found during any period of the infection in the chick. On the contrary, there was in chicks with a patent infection a 33 per cent increase above normal in terms of milligrams of ascorbic acid per adrenal gland (table 1) resulting from the adrenal hypertrophy associated with the malarial infection (Nadel *et al.*, 1949).

The fact that chicks require no ascorbic acid in the diet may account for our failure to observe a decrease in adrenal ascorbic acid in the malaria infected chicks in contrast

TABLE 4

Ascorbic acid concentrations in the adrenal glands of uninfected chicks fed or starved for 5 days

GROUP	AGE AT SACRIFICE	DIET PRIOR TO SACRIFICE	ADRENALS	
			No. pairs pooled	Ascorbic acid*
	days			
A	24	Starting mash and water *ad libitum*	5	1.43
			5	1.12
B	29	Starting mash and water *ad libitum*	5	1.32
			5	1.01
C	29	No food or water for 5 days	5	1.49
			5	1.49
			4	1.28

* Milligrams of ascorbic acid per gram of fresh adrenal tissue.

to the findings in monkeys. It was first thought that the observed adrenal hypertrophy might be an adjustment made by the chick to an altered ascorbic acid metabolism during the course of the infection. However, preliminary observations indicate that infected chicks receiving supplementary ascorbic acid showed a degree of adrenal hypertrophy comparable to those not receiving the vitamin. Data in figure 1 and table 3 show that the course of the infection was not altered by daily administration of ascorbic acid.

Long (1947) found lowered adrenal ascorbic acid values in rats, which also require no dietary ascorbic acid, when subjected to a variety of stress agents including starvation. In the chick, however, near-fatal starvation resulted in no reduction in adrenal ascorbic acid concentration.

The complete interpretation of the observed phenomena on chicks infected with *P. gallinaceum* is hardly possible at this time. Additional experiments are needed to determine whether the failure to observe changes in adrenal ascorbic acid concentrations is the result of (a) *P. gallinaceum* infections having no effect on the ascorbic

acid metabolism of the chick or (b) a masking of the effect of *P. gallinaceum* infections on the ascorbic acid metabolism of the host by the independent ability of the chick to synthesize this vitamin.

SUMMARY

The adrenal ascorbic acid concentration in the chick, an animal which requires no dietary ascorbic acid, was unchanged during the course of a blood-induced malarial (*P. gallinaceum*) infection. There was an absolute increase in ascorbic acid per gland proportional to the degree of adrenal hypertrophy observed during the course of the infection.

Daily injections of 10 mg. of ascorbic acid had no effect upon the course of parasitemia, day of death, or degree of adrenal hypertrophy.

Withholding of food and water from chicks for 5 days resulted in no change in the adrenal ascorbic acid concentration.

ACKNOWLEDGMENT

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REFERENCES

Ball, E. G., Anfinsen, C. B., Geiman, Q. M., McKee, R. W., and Ormsbee, R. A. 1945. *In-vitro* growth and multiplication of the malaria parasite *Plasmodium knowlesi*. Science, **101**: 542–544.

Biester, H. E., and Schwarte, L. H. 1948. Diseases of Poultry. 2nd edition, pp. 195–196, The Iowa State Press, Ames, Iowa.

Gerdjikoff, I. 1939. Uber die Blutbeschaffenheit bei Malaria unter Vitamin C-Belastung. Klinische Wochenschrift, **18** (36): 1214–1217.

Hawk, P. B., Oser, B. L., and Summerson, W. H. 1947. Practical Physiological Chemistry, 12th edition, page 1137, The Blakiston Company, Philadelphia, Pennsylvania.

Krishnan, K. V. 1940. Tropical Diseases Bulletin, **37**: 744–745.

Long, C. N. H. 1947. The relation of cholesterol and ascorbic acid to the secretion of the adrenal cortex. Recent Progress in Hormone Research, **1**: 99–122.

Lotze, H. 1938. Clinical experimental investigations in benign tertian malaria. Tropical Diseases Bulletin, **35**: 733–734.

McKee, R. W., and Geiman, Q. M. 1946. Studies on malarial parasites. V. Effects of ascorbic acid on malaria (*Plasmodium knowlesi*) in monkeys. Proceedings of the Society for Experimental Biology and Medicine, **63**: 313–315.

McKee, R. W., Cobbey, T. S., Jr., and Geiman, Q. M. 1947. Observations on the action of ascorbic acid in adrenal cortical function. Federation Proceedings, **6** (1): 276.

Mohr, W. 1943. Malaria and assimilation of Vitamin C. Tropical Diseases Bulletin, **40**: 13–14.

Nadel, E. M., Taylor, D. J., Greenberg, J., and Josephson, E. S. 1949. Adrenal hypertrophy in chicks infected with *Plasmodium gallinaceum*. J. Nat. Mal. Soc., **8**: 70–79.

Reniger-Arshiva, M. L. 1944. Vitamin C in the placenta and in the foetal organs in malaria. Tropical Diseases Bulletin, **41**: 919–920.

Sorce, S., and Mutolo, U. 1946. L'Ascorbinemia nell' infezione malarica. Bollettino della Societa Italiana di Biologia Sperimentale, **22**: 399–400.

Wozonig, H. 1945. Die Vitamin C—Gehalt der Cerebrospinalflussigheit bei naturlicher Malaria. Zeitschrift Immunitatsforschung und Experimentelle Therapie, **105**: 411–416.

LABORATORY STUDIES ON THE RESISTANCE OF *ANOPHELES QUADRIMACULATUS* TO DDT AND OTHER INSECTICIDES[1]

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Development of resistance in some insect populations repeatedly exposed to insecticides presents the possibility of the loss of effectiveness of many chemicals which have been most useful in the control of certain insects. Quayle (1943) reviewed the literature and discussed in detail the resistant strains of seven insect species, namely: (1) the San Jose scale, *Quadrispidiotus perniciosus* (Comst.), to lime-sulfur spray (Melander, 1914); (2) the California red scale, *Aonidiella aurantii* (Mask.), to HCN fumigation (Quayle, 1916); (3) the black scale, *Saissetia oleae* (Bern.), to HCN fumigation (Quayle, 1916); (4) the young larva of the codling moth, *Carpocapsa pomonella* (Linn.), to arsenical and other sprays (Hough, 1928); (5) the citricola scale, *Coccus pseudomagnoliarum* (Kuw.), to HCN fumigation (Quayle, 1938); (6) the larva of the primary screw worm, *Cochliomyia americana* C&P to phenothiazine (Knipling, 1942); and (7) the citrus thrips, *Scirtothrips citri* (Moult.), to tartar emetic-sucrose spray (Boyce et al., 1942).

Mosna (1948) quoted further work on resistant strains: (1) the blue cattle tick, *Boophilus annulatus* var. *decoloratus* (Koch), to arsenical sprays (Whitnall and Bradford, 1947); (2) the house fly, *Musca domestica* (Linn.), to DDT (Missiroli, 1947 and 1948), (Saccà, 1947) and (Wiesmann, 1947); and described his own work on a strain of the mosquito, *Culex pipiens* var. *autogenicus* resistant to DDT. Recent work in this country has shown the existence of resistant strains of the house fly to DDT (Lindquist and Wilson, 1948), (Wilson and Gahan, 1948) and (Barber and Schmitt, 1948) and to benzene hexachloride (Blickle et al., 1948).

A study was undertaken to determine the possible development of a resistant strain of *A. quadrimaculatus* mosquitoes and to determine the extent of such resistance, if any, to a variety of residual insecticides, since the resistance of insects in some cases has been manifested to specific insecticides (Knipling, 1942), (Mosna, 1948), and (Bettini and Barachini, 1948), to closely related chemical compounds (Barber and Schmitt, 1948), and to insecticides in general (Wilson and Gahan, 1948) (Blickle et al., 1948).

METHOD

An insectary-reared strain of *A. quadrimaculatus* mosquitoes was used in this investigation. Pupae, reared at 80° F. and 70 per cent relative humidity, were placed in emergence cages, where a few adults emerged at the end of 12 hours and the majority within 36 hours.

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To avoid the introduction of offspring of non-exposed males into the breeding strains of subsequent generations, the sexes were separated, by means of an air aspirator, within 24 hours after emergence. After separation about 60 adults of each sex were placed in separate holding cages previously described (Simmons et al. 1945) and allowed to feed on a 1:10 honey-water solution for 24 hours before further handling.

The mosquitoes of each sex were then subjected to DDT deposits in an exposure chamber (Fay et al., 1947), consisting of a wooden framework into which four 3- by

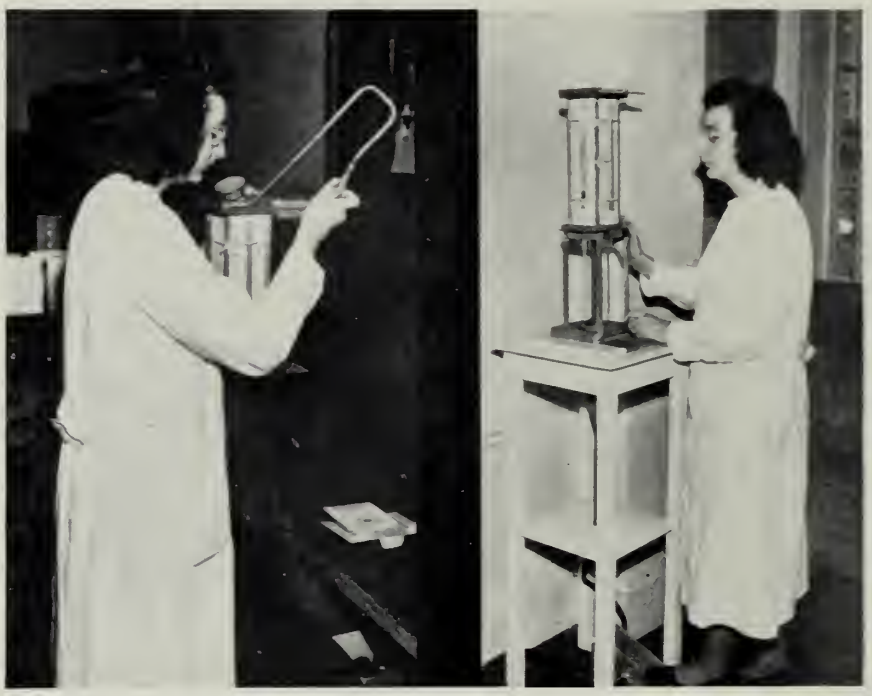

FIG. 1A. (Left). Transfer of mosquitoes by means of air currents from glass transfer cage into exposure chamber in preparation for testing.

FIG. 1B. (Right). Removal of the mosquitoes from exposure chamber by air currents into holding cage. Test insects supplied with food and held 24 hours to determine mortality.

12-inch DDT-treated plywood panels were fitted and braced to form the sides of the chamber (figure 1). Circular openings in each end of the framework permitted the introduction and removal of the insects.

The test panels were sprayed as a single flat surface which allowed a more uniform application of the spray. A DeVilbiss atomizer was used with 30 psi air pressure and 4 mls. of a 5- or 10-per cent-DDT xylene emulsion was applied to each set of panels to give deposits of 200 and 400 mgs. DDT per square foot respectively. After spray application, the panels were allowed to aerate three days before they were used.

The mosquitoes were transferred by air currents from a holding cage into a glass

transfer cage. The transfer cage was then placed over an air-blast mechanism and the exposure chamber, with both doors open to permit free passage of air, was fitted on top of the transfer cage. After the metal door of the transfer cage was opened, a slight gust of air, released by means of a pedal on the air-blast mechanism, gently lifted the mosquitoes into the exposure chamber. The panel number and the length of the exposure period were recorded.

During the exposure, the chamber was entirely darkened to eliminate light attraction and placed on one side, as it had been found previously that mosquitoes would alight more satisfactorily on the treated surfaces in this position. At the end of the exposure period, the mosquitoes were gently blown into a screen wire holding cage. A small pad of cotton, containing a honey-water solution, was placed on the holding cage which was then held in a constant-temperature and humidity room, and after 24 hours the mortality was recorded.

If the 24-hour mortality exceeded 50 per cent, the surviving mosquitoes were placed in a colony cage for the production of the next generation and were allowed to mate and lay eggs (with less than 50 per cent mortality, the survivors were re-exposed). Larvae were reared from these eggs, and the pupae placed in emergence cages. The emerging adults were designated as the second generation, and the segregation and exposure technique previously described was repeated. Adult mosquitoes of the third, fourth, and fifth generations were obtained by this same technique. The adults of the fifth generation were not exposed to DDT, but all of the mosquitoes were given an opportunity to mate and to lay eggs. The adults produced from these eggs were designated as the sixth generation.

In the final evaluation of comparative resistance, adults of all six generations were exposed in successive tests under similar conditions to each of four sets of panels containing equal amounts of DDT residual deposits. Further evaluations were made of comparative resistance to residual deposits of other insecticides. The 24-hour-mortality percentages were subjected to angular transformations (Bliss, 1937) and the significance of the difference between the average mortality of the first generation and of each successive generation was determined by Students' t-test.

SPECIAL PROBLEMS IN TECHNIQUE

If mating occurred before a selective kill by exposure to DDT residues had been made, it was possible that a female which survived the exposure might have been fertilized by a weak male which was later killed by the exposure to DDT, and the offspring, in this case, would result from one DDT-resistant parent only. Thus, it was necessary to separate the sexes prior to the time of mating. An indirect method, based on the assumption that unfertilized females would not oviposit, was used to determine the interval of time between emergence and mating. Adults were given opportunity to mate for intervals of 24, 48, 72, and 96 hours after emergence. The males were then removed from each cage, the females were given blood meals, and the rate of oviposition in the following 14-day period was recorded. Normal oviposition occurred when the sexes were together for 72 to 96 hours, a few eggs were laid when the adults were together for 48 to 72 hours, but no eggs were laid when the adults were together for the shorter intervals. From the above obser-

vations, it was assumed that no mating had taken place within the first 24 hours after emergence, and the sexes were usually separated within this period in the experimental technique.

It was found necessary to devise some method whereby the emergence of adults could be delayed over the week end so that more pupae could be utilized for testing. Refrigeration of the pupae at 40° F. for periods of 24, 48, and 72 hours was tried. Satisfactory adult emergence, after a delay of 24 hours, was obtained, but not after longer periods of exposure to cold.

TABLE 1

Average 24-hour mortality (per cent) of 2-day-old, adult male and female Anopheles quadrimaculatus *of successive generations after exposure periods of definite length to deposits of 400 mg. DDT per square foot on plywood panels*

GENERATION	SEX	RANGE OF EXPOSURE PERIODS	NUMBER OF TESTS	AVERAGE MORTALITY
		minutes		*per cent*
1	Male	5– 20	428	76
	Female	25– 60	983	68
2	Male	8– 30	246	71
	Female	45– 75	313	65
3	Male	8– 35	249	67
	Female	60– 85	277	61
4	Male	8– 40	237	66
	Female	60– 95	248	62
5	Male	8– 45	174	64
	Female	90–105	192	62
1–5	Male	5– 45	1334	70
	Female	25–105	2013	65

Fay et al. (1947) reported that male mosquitoes are killed with shorter exposure periods to DDT than are the females. The residual effectiveness of DDT depends upon the concentration and age of the deposits and the length of the exposure periods. The desired 66 per cent mortality for each sex over the 8-month study period was produced by using a few series of test panels and extending the length of exposure periods (table 1) as the residual deposits aged, so as to reduce panel preparation to a minimum.

In the insectary technique, only six per cent of the resistant strain of *A. quadrimaculatus* eggs set finally resulted in adults actually usable for a selective kill. With this loss, selective mortality levels of 80 per cent or more were too high to permit the establishment of active productive colonies. With a selective level of 66 per cent mortality, it was necessary to supplement the colony of each generation with simi-

larly exposed adults four days of each week to secure adequate egg production for continuance of the study.

When approximately 125 adults per day, surviving the DDT exposure, were introduced four days each week into a colony cage, about 22 days were required for the production of the next generation of adults. This period, corresponding to the life cycle of the mosquito, was divided as follows: three days from adult emergence to mating, seven days from mating to oviposition, nine days from egg to pupae, two days from pupation to adult emergence, and one day before the adults were exposed to DDT residues. A period of four months was required to build up all six generations to the point where the colonies produced enough adults for colony maintenance and for comparative resistance studies.

TABLE 2

The mean 24-hour mortalities (per cent) from 12 to 16 replications using approximately sixty, 1-day-old, adult female A. quadrimaculatus *mosquitoes exposed for definite periods to deposits of 200 mg. per square foot of different insecticides*

CHEMICAL	EXPOSURE PERIOD	REPLICATES PER GENERATION	MEAN 24-HOUR MORTALITY (PER CENT)					
			Generation*					
			1	2	3	4	5	6
	minutes							
DDT	30	12	43.9	26.0	24.7	25.7	24.0	40.2
DDT	50	14	53.0	35.3	32.3	33.4	35.9	53.5
Chlordan	30	16	86.4	87.5	86.0	87.5	89.3	88.2
Benzene Hexachloride	5	12	24.3	25.0	22.2	26.0	21.1	24.8
118	5	12	53.4	49.1	50.6	49.8	50.9	53.9

* Adults of first and sixth generations from eggs deposited by females not exposed to DDT, other generations from eggs deposited by exposed females.

RESULTS

For the study of comparative resistance of the six generations, adult female mosquitoes were exposed to panels containing deposits of 200 mg. per square foot of the following insecticides: DDT, chlordan, benzene hexachloride, insecticide 118[2], dichloro-diphenyl dichloroethane (DDD), and methoxychlor. For each of the six generations, 12 to 16 replications, each using approximately sixty 1-day-old females, were made to test each chemical, and the 24-hour mortalities (per cent) were recorded. The means of the 24-hour mortality per generation are listed in table 2 for DDT and those insecticides of markedly different chemical structure, namely chlordan, benzene hexachloride, and insecticide 118; whereas table 3 contains the results with DDT and closely allied chemicals, namely DDD (TDE) and methoxychlor. Since some of the insecticides killed all of the mosquitoes at the longer exposure periods, it was necessary to shorten the period in some cases.

A statistical test of significance (t-test) of the difference between the mean mortality of the first generation and that of each subsequent generation was made follow-

[2] A proprietary insecticide furnished by the Julius Hyman Co., Denver, Colo.

ing an angular transformation of the percentage values. With 30-minute exposures to DDT, the differences between the first generation and the second, third, fourth, and fifth generations respectively exceeded the 5 per cent level of significance. There was no significant difference between the mean mortality of the first and sixth generations, however.

The study was then repeated with 50-minute exposures to DDT to compare the results between generations at a higher rate of mortality. These results presented the same relative picture of resistance.

Tests with 30-minute exposures to a series of panels containing 200 mgs. chlordan per square foot failed to show a statistically significant difference between the mean mortalities of the first and any subsequent generation. The 5-minute exposures to deposits of 200 mgs. benzene hexachloride per square foot (10 per cent gamma-isomer) indicated no significant difference between the mean mortalities of the six

TABLE 3

The mean 24-hour mortalities (per cent) from 12 to 14 replications using approximately sixty, 1-day-old, adult female A. quadrimaculatus *mosquitoes exposed for 50 minutes to deposits of 200 mg. per square foot of DDT and closely allied insecticides*

CHEMICAL	EXPOSURE PERIOD	REPLICATES PER GENERATION	MEAN 24-HOUR MORTALITY (PER CENT)					
			Generations*					
			1	2	3	4	5	6
	minutes							
DDT	50	14	53.0	35.3	32.3	33.4	35.9	53.5
DDD	50	12	21.2	20.5	20.3	21.1	23.8	19.2
Methoxychlor	50	12	48.5	32.6	32.3	30.1	33.6	50.5

* Adults of first and sixth generations from eggs deposited by females not exposed to DDT, other generations from eggs deposited by exposed females.

generations. A similar picture of the mean mortalities of different generations was also given by 5-minute exposures to deposits of 200 mgs. per square foot of insecticide 118.

Interesting results were obtained when chemicals closely allied to DDT were tested. Fifty-minute exposures to a series of panels containing 200 mgs. DDD per square foot failed to show a statistically significant difference between the mean mortalities of the first and any subsequent generation. However, when 50-minute exposures to deposits of 200 mgs. methoxychlor per square foot were made, the differences between the mean mortality of the first generation and that of the second, third, fourth, or fifth generation exceeded the 5-per cent level of significance. There was no significant difference between the mean mortality of the first and sixth generations.

DISCUSSION

Tests with DDT Deposits. The increase in resistance to DDT was produced by the first selection of the adults and did not change materially with subsequent selections over four generations. When the adults were not selectively killed, as in the

fifth generation, then their offspring reverted to the degree of resistance shown by the untreated strain. These observations can be interpreted in the light of genetic studies as the result of chance selection, of genetic differences, either naturally occurring or induced, or of somatic modification of the mosquitoes and the possible cause of the resistance conjectured.

The survival of given individuals after exposure to DDT might have been due completely to chance factors, e.g. a combination of optimum conditions in the rearing technique, but the offspring of such survivors would not be expected to differ materially in resistance from the original population. This possible explanation of the selective exposure to DDT can be discarded as the second generation adults did show an increased resistance to DDT.

The genetic composition of the survivors might have differed from that of the general population, e.g. if a genetic difference existed in the population which was of minor importance in the normal environmental conditions, or if such a genetic difference was induced by a chemical exposure (cf. McCarty, 1946), the introduction of the selective kill by DDT might favor the proportion of the population of one genetic composition and eliminate a large proportion of the less-resistant individuals. The population composition of the next generation, in this case, would be expected to differ from the original population in the proportion of the resistant mosquitoes, and the extent of the difference would depend on the exact genetic mechanism responsible for the difference. With the assumption that the adult mosquitoes mate at random and that the factor determining resistance is inherited independently of other factors, it can be shown mathematically that the proportion of mosquitoes with the factor for resistance would increase from generation to generation until a stock homozygous for resistance was produced. Since no increase was noted in four generations after the first selection, and since reversion to the stock condition was immediate after cessation of selective kill, this explanation does not seem to hold.

There is at least one modification of this explanation that would not be ruled out on the basis of the experimental evidence. Since only six per cent of the eggs laid by the exposed adults resulted in test adults for the next generation, this mortality represents a second selective kill in the experiment. If one assumes that 15,000 eggs produced only 1,000 adults, and that 700 of these adults were selectively killed by DDT deposits to give only 300 adults which in turn produced 15,000 eggs for the next generation, it is clear that the selective process may be divided into two parts, x equalling the selection of the rearing technique and y equalling the selection of the DDT exposure. It is possible that selection y might affect adults with the normal gene A and the mutant gene a to different degrees and that the 15,000 eggs might represent a new population ratio of A to a differing from that in the original population. Then the x selection might have an inverse effect on A and a to produce the original population ratio in the resulting 1,000 adults of the next generation. Smith (1941) noted this possible exception, but a special hypothesis concerning the nature of selection x would be required to explain the reversion to normal resistance which appeared to be complete with one omission of the DDT exposure. Actually, the mortality of selection x did not appear to differ in the production of the first and subsequent generations.

The work of Jollos (1921), Sonneborn (1943) and Kimball (1947) demonstrated that if certain protozoa were exposed to sublethal concentrations of various toxins, the survivors showed increased tolerance to concentrations of the toxins lethal to non-conditioned individuals of the species. The increased tolerance was transmitted through a few asexual generations but disappeared with the first fertilization. The induced modification (*Dauermodificationen*) was due to cytoplasmic changes only. This last explanation appears to fit the experimental data of the present study since the acquired resistance was maintained in successive generations only so long as the selective exposure to DDT was continued with each generation of adults previous to mating. It is possible that the DDT was capable of inducing somatic changes in the sperm or egg cells of the adult mosquitoes and that these changes were carried only through the next generation. This explanation is favored over the modification noted above because the return to a normal degree of resistance appeared to be complete in the first generation after omission of the selective kill. This would be expected in the case of *Dauermodificationen*, whereas it requires special explanation in the noted modification.

Tests with Deposits of Other Insecticides. Since the acquired resistance to DDT, as exhibited by the second through fourth generations of mosquitoes used in the present study, was not extended to other insecticides, namely chlordan, benzene hexachloride, insecticide 118, and DDD, the proposed induced somatic change appears to be specific. In view of the susceptibility of the DDT-resistant mosquitoes to DDD and their resistance to the methoxy analogue of DDT, it appears that the induced resistance might involve the bridge of the toxic molecules rather than the substituted phenyl groups. Additional tests with compounds having modified bridge and ring grouping should be investigated to substantiate this postulation.

General Practical Application of the Study. Since there is a fundamental capacity for mutation in each species and since all mutational types repeatedly appear in large populations (Smith, 1941), the authors believe that the failure to produce any permanent resistance in adults of *A. quadrimaculatus* mosquitoes in the present study involving a population of 750,000 adults may well indicate that such a mutation, if it exists, must be quite rare, or that if such mutants have arisen in the present study, they have been submerged by the rearing selection. In nature, the environmental selection is more severe due to additional hazards, e.g. desiccation, predators, adverse water actions, etc., and this selection coupled with the fact that relatively low proportions of a wild population come in contact with DDT would minimize the probable development of a stable mutant resistant strain of the insect in question.

SUMMARY

A technique is described for producing, by exposure to DDT residual deposits, a 66-per cent selective kill of each sex of adult *Anopheles quadrimaculatus* mosquitoes. The selective kill is applied to adults of four successive generations but not to the adults of a fifth and sixth generation in a study of the production of a DDT-resistant strain of the species.

By means of an indirect method, it is shown that a 48-hour interval exists between the emergence of the adults and the act of mating. Refrigeration of pupae for ap-

proximately 24 hours at 40° F. delays adult emergence 24 hours but does not materially reduce the rate of emergence, whereas longer periods of refrigeration adversely affect emergence.

Studies on the comparative resistance of the various generations to DDT demonstrate a significant increase in resistance after the first selective kill. No additional increase in resistance is evident through the next three selective exposures, and with the omission of selective exposure, the succeeding generation reverts to the stock resistance.

The DDT-induced resistance does not extend to the following insecticides: chlordan, benzene hexachloride, insecticide 118, and dichlorodiphenyldichloroethane. Tests with the methoxy analogue of DDT presents the same picture of resistance as the DDT exposures.

The genetic possibilities of the resistance are discussed, and the production of an induced, non-genetic mutation, possibly specific for the modifications of the bridge portion of the molecule, is proposed.

REFERENCES

Barber, G. W. and Schmitt, J. B. 1948. House flies resistant to DDT residual sprays. N. Jersey Agr. Exp. Sta. Bull., **742:** 1–8.

Bettini, S. and Barachini, B. 1948. Primi risultati della lotta con l'octaklor ed il gammaesano contro le mosche domestiche resistenti al DDT. Riv. Parass., **9:** 85–91.

Blickle, R. L., Capelle, A., and Morse, W. J. 1948. Insecticide resistant house flies. Soap & San. Chem., **24:** 139, 141, 149.

Bliss, C. 1937. The analysis of field experimental data expressed in percentages. (In Russian) Plant Protection, **12:** 67–77.

Boyce, A. M., Persing, C. O., and Barnhart, C. S. 1942. The resistance of citrus thrips to tartar emetic-sucrose treatment. J. Econ. Ent., **35:** 790–1.

Fay, R. W., Simmons, S. W., and Clapp, J. M. 1947. Extended laboratory investigations on the toxicity of DDT residues to adults of *Anopheles quadrimaculatus.* Pub. Health Rep., **62:** 149–158.

Hough, W. S. 1928. Relative resistance to arsenical poisoning of two codling moth strains. J. Econ. Ent., **21:** 325–29.

Jollos, V. 1921. Experimentelle protistenkunde. I. Untersuchungen über variabilitat und vererbung bei Infusorien. Arch. Protist., **43:** 1–222.

Kimball, R. F. 1947. The induction of inheritable modification in reaction to antiserum in *Paramecium aurelia.* Genetics, **32:** 486–99.

Knipling, E. F. 1942. Acquired resistance to phenothiazine by larvae of the primary screw worm. J. Econ. Ent., **35:** 63–4.

Lindquist, A. W. and Wilson, H. G. 1948. Development of a strain of house flies resistant to DDT. Science, **107:** 276.

McCarty, M., Taylor, H. E., and Avery, O. T. 1946. Biochemical studies of environmental factors essential in transformation of pneumococcal types. Cold Spring Harbor Symp. Quant. Biol., **9:** 177–183.

Melander, A. L. 1914. Can insects become resistant to sprays? J. Econ. Ent., **7:** 167–73.

Missiroli, A. 1947. Riv. Parass., **8:** 141–169. (From Mosna).

Missiroli, A. 1948. La lotta contro gl'insetti domestici. Annali Sanit. Pub., **9:** 4–13.

Mosna, E. 1948. *Culex pipiens autogenicus* DDT-resistenti e loro controllo con octa-klor e esaclorocicloesano. Riv. Parass., **9:** 19–25.

Quayle, H. J. 1916. Are scales becoming resistant to fumigation? Cal. J. Agr., **3:** 333–4, 358.

Quayle, H. J. 1938. Development of resistance to hydrocyanic acid in certain scale insects. Hilgardia, **11:** 183–210.

Quayle, H. J. 1943. The increase in resistance in insects to insecticides. J. Econ. Ent., **36:** 493–500.

Saccà, G. 1947. Riv. Parass., **8:** 127–8 (From Mosna).

Simmons, S. W., and Staff. 1945. Techniques and apparatus used in experimental studies on DDT as an insecticide for mosquitoes. Pub. Health Rep. Supplement, **186:** 3–21.

Smith, H. S. 1941. Racial segregation in insect populations and its significance in applied entomology. J. Econ. Ent., **34:** 1–13.

Sonneborn, T. M. 1943. Development and inheritance of serological characters in variety 1 of *Paramecium aurelia*. Genetics, **28:** 90.

Whitnall, A. B. and Bradford, B. 1947. An arsenic-resistant tick and its control with gammexane dips. Bull. Ent. Res., **38:** 353–372.

Wiesmann, R. 1947. Schweiz. Ent. Gesell., **20:** 484–504. (From Mosna).

Wilson, H. G. and Gahan, J. B. 1948. Susceptibility of DDT-resistant house flies to other insecticidal sprays. Science, **107:** 276–7.

GIEMSA, AZURE B TYPE, RECOMMENDED FOR MALARIA

At the April 16 meeting of the Biological Stain Commission the Board of Trustees voted that hereafter Giemsa stain will be certified in two varieties, namely "Giemsa stain, Azure B Type, for malaria and blood work" and "Giemsa stain, Azure A type, for hematology and bacteriology."

The Azure B type closely resembles tinctorially and spectroscopically the Grübler and Hollborn Giemsa stains of the 1930's and is the variety especially recommended to give faintly greenish blue tint to parasite cytoplasm which contrasts well with the grayish or greenish blue background of the thick film stained at pH 7.0. This type of stain is particularly suited for parasite studies in either thick or thin blood films and is recommended by many malariologists.

The Azure A type gives darker red chromatin stains, grayer or more violet blue lymphocyte cytoplasm, and perhaps somewhat heavier staining of micro-organisms. Its useful life is probably shorter under average tropical storage conditions. It is preferred by many American hematologists.

ANOPHELES QUADRIMACULATUS ACTIVITY PATTERNS IN THE LABORATORY ON UNTREATED AND DDT-TREATED SURFACES[1]

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Many conflicting statements have appeared in the literature concerning the actual contact period with DDT deposits necessary for lethal effects on mosquitoes. Continuous exposure to fresh deposits of 200 mg. DDT per square foot (Knipling, 1945) gave 16 per cent knock-down in 1 hour, 64 per cent knock-down in 2 hours 97 per cent knock-down in 3 hours, and 100 per cent knock-down only after 4 hours of exposure. Short exposure periods of 15 and 30 minutes gave 24-hour mortalities of 46 and 56 per cent respectively. Five-minute contacts with new deposits of 200 mg. DDT per square foot (Metcalf, *et al.*, 1945) were reported to give 24-hour mortality of 20 per cent. Exposures of 28, 35, and 54 minutes to fresh DDT deposits (Fay, *et al.*, 1945) were necessary to produce 24-hour mortalities of 80, 90, and 100 per cent respectively. All of these observations were based on the laboratory confinement of adult *A. quadrimaculatus* mosquitoes in various types of exposure chambers having the majority of the available resting surface treated with DDT deposits.

In contrast to these observations, adults of *A. gambiae* and *A. funestus* after 1-hour exposures on 5-month-old deposits of 200 mg. DDT per square foot (MacInnes, 1947) showed 100 per cent mortality within 3 hours, while 5-minute exposures gave 73 per cent mortality in 12 hours. Adults of *A. albimanus* (Elmendorf, *et al.*, 1946) demonstrated 100 per cent mortalities in 5 hours from 4 instantaneous contacts with screen wire treated with solutions of 200 mg. DDT per square foot. To secure comparable results with dry DDT deposits, 2 to 5 minutes of exposure were necessary. A total kill of *A. gambiae* was obtained 9 hours following a 60-second confinement in aspirator tubes coated with DDT (Kartman and da Silveira, 1946), whereas 10 to 24 hours elapsed before a 100 per cent kill was obtained following a 30-second contact, and a 5-second contact gave 97 per cent mortality at 10 to 24 hours after exposure.

Other observations (Kennedy, 1947) demonstrated that the DDT-deposits showed repellent and excitant effects on adults of *Aedes aegypti* and *Anopheles maculipennis atroparvus*. It was stated that adults of these species may show all signs of DDT-toxicity and still recover. Field observations (Hocking, 1947), (Thompson, 1947) gave evidence that the adult mosquitoes *A. gambiae* and *A. funestus* were stimulated by the irritant properties of DDT deposits to leave treated houses before they had received a lethal dose.

In view of these observations, the following study was made to determine (1) the

[1] From Communicable Disease Center, Technical Development Division, Savannah. Georgia.

length of time an adult female *A. quadrimaculatus* mosquito had to remain in actual contact with DDT deposits in order to receive a lethal dose and (2) the behavior pattern of this species on untreated and DDT-treated surfaces.

MATERIALS AND METHODS

In the experimental method, each observation utilized one 3-day-old, adult female mosquito. The insect was transferred from an emergence cage to a glass tube, $1\frac{1}{2}$ inches in diameter, equipped with a plexiglass plunger. The tube was placed in a holding rack with the open end covered by a panel designated as the test panel whether treated or untreated (figure 1) and the plunger was pushed to within 1 inch

FIGURE 1. Method and equipment for the study of the mosquito behavior patterns including the observation chamber, test panel, data sheet, and individual holding containers.

of the panel. From the time of the initial contact with the test panel all observations were timed with a stopwatch and notations were made concerning (a) the number of movements on and off the test surface, (b) the number of points of contact, i.e., legs and proboscis, (c) the type of general activity, i.e., resting, walking, flying, probing, and cleaning, and (d) progressive physiological manifestations of DDT toxicity. At the end of a definite observation period, the insect was placed in a clean glass container, supplied with food and water, and observed after 24, 48, 72, and 96 hours.

The two types of test surfaces selected were plywood and filter paper. Untreated surfaces and treated surfaces containing deposits of 200 mg. DDT per square foot, 1 to 10 days old, were tested. Treated surfaces were inspected with a microscope to check presence of DDT crystals in each case before use in tests. No attempt was made to control the movements of the test insect in the chamber and 25 replications

were made on each type of treated surface for total observation periods of 20 and 40 minutes, respectively, with 20 additional replications at 60 minutes on both the treated and untreated surfaces.

To facilitate the discussion and graphical presentation of the data, the continuous observations over the entire exposure period have been considered in 2-minute intervals after the initial contact with the test surface.

RESULTS

Although the test panel furnished only 26 per cent of the available resting surface in the observation chamber, preferential selection by the adult mosquitoes of wood or filter-paper surfaces to those of plexiglass or glass resulted in satisfactory

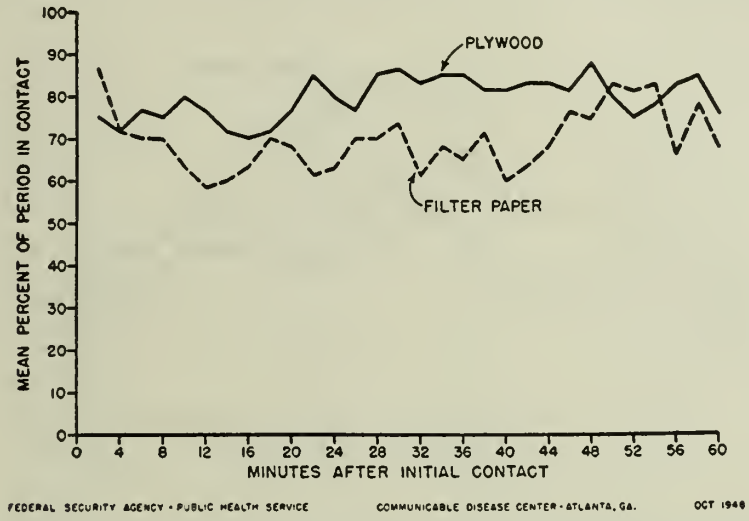

FIGURE 2. Mean percent of each 2-minute period after the initial contact with untreated surfaces (plywood or filter paper) spent on the surface by adult female *A. quadrimaculatus* mosquitoes.

contact with the test surfaces. Since the mosquitoes had ample room for sustained flight and since no effort was made to control their activities, the contact time with the test surface varied with the individual exposures. The average per cent of contact time with the test surface for each 2-minute period after the initial contact, based on all replications, is presented graphically for untreated surfaces (figure 2) and for DDT-treated surfaces (figure 3). The adult mosquitoes remained in contact a distinctly higher per cent of time where untreated plywood was used as the test surface than where untreated filter paper was used. A DDT treatment of these surfaces, however, produced an inverse relationship in the contact time, with the filter paper as the preferred surface. This relationship is interesting in that pretreatment evaluation of preferred resting surfaces cannot be translated safely into posttreatment values. On the untreated test surfaces (figure 2), the mean time of contact during any 2-minute period after the initial contact was quite constant and showed a gradual increase of about 10 per cent over the 1 hour observa-

tion period. On the DDT-treated surfaces (figure 3), however, the mean time of contact during successive 2-minute periods shows a decided decrease for the first 12 minutes after the initial contact, as the excitant effect of DDT is the predominant toxic manifestation. During the period from 12 to 28 minutes the mean contact time shows increasing values as the paralytic action of DDT forced the insects to cling to the surface for support. At more than 28 minutes, the mean contact time values again decrease as a result of the number of mosquitoes showing complete paralysis, or knock-down. The mean number of changes on and off the test panels in each 2-minute period is given for the filter paper and plywood surfaces (table 1). The greater number of changes on the untreated filter-paper surface indicates that it is not as desirable a resting surface as the untreated plywood. After the applica-

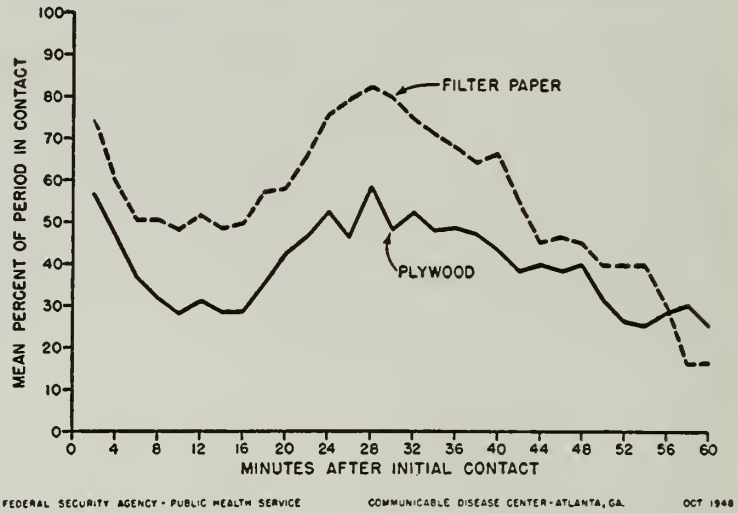

FIGURE 3. Mean percent of each 2-minute period after the initial contact with surfaces (plywood or filter paper) containing deposits of 200 mg. DDT per square foot spent on the surface by adult female *A. quadrimaculatus* mosquitoes.

tion of DDT this preference is exhibited only during the first 20 minutes after the initial DDT contact and after this period there is no marked difference in the number of changes between the two types of surfaces. This may indicate either (a) the mosquitoes are no longer able to detect surface differences or (b) with the onset of paralysis the insect's need for support is a more important factor than the type of resting surface.

Combined results on the untreated surfaces (figure 4) show the mean number of changes in each 2-minute period decreased gradually from 1.45 to 0.8 times over the 1-hour observation period. With DDT-treated surfaces, however, the mosquitoes moved more frequently than normal during the first 28 minutes after the initial contact and then less frequently. On the basis of the experimental evidence of these studies, the mean number of times that a mosquito will move off and onto a treated surface during intervals of 2, 10, and 20 minutes after the initial contact and during the subsequent 20–40 and 40–60 minutes have been caluculated (table 2).

Since the only changes possible are onto or off of the surface and since two changes must alternate, the mean number of changes divided by two gives an indication of the number of times a mosquito could be expected to leave the surface and thus

TABLE 1

Mean number of moves on and off of untreated and DDT-treated surfaces of plywood and filter paper shown by adult female A. quadrimaculatus mosquitoes at 2-minute intervals after the initial contact

INTERVAL AFTER INITIAL CONTACT	MEAN NUMBER OF CHANGES PER PERIOD			
	Plywood		Filter Paper	
	Untreated	DDT-treated	Untreated	DDT-treated
minutes				
0– 2	1.4	2.09	1.5	1.86
2– 4	0.85	2.04	2.05	3.96
4– 6	0.95	2.0	1.05	3.22
6– 8	0.75	2.64	1.15	3.24
8–10	1.1	2.2	1.2	2.89
10–12	1.1	2.13	0.9	3.01
12–14	1.0	2.06	1.35	2.51
14–16	0.75	1.83	1.5	2.51
16–18	0.8	2.07	1.25	2.5
18–20	0.9	1.47	1.05	1.86
20–22	0.8	1.42	1.2	1.42
22–24	0.95	1.16	0.9	1.33
24–26	0.8	1.16	1.15	0.89
26–28	0.65	0.78	0.9	0.89
28–30	0.55	0.47	1.2	0.44
30–32	0.75	0.73	0.75	0.64
32–34	0.5	0.67	1.15	0.51
34–36	0.25	0.51	1.85	0.47
36–38	0.45	0.53	1.2	0.4
38–40	0.85	0.33	1.6	0.22
40–42	0.45	0.05	0.6	0.1
42–44	0.45	0.25	0.95	0.1
44–46	0.5	0.25	0.75	0.20
46–48	0.55	0.25	1.2	0.
48–50	0.4	0.15	1.0	0.05
50–52	0.45	0.	1.1	0.15
52–54	0.45	0.2	1.1	0.1
54–56	0.5	0.15	1.1	0.15
56–58	0.45	0.2	0.95	0.2
58–60	0.45	0.05	1.15	0.15

might possibly escape from further lethal effects. Since each time the insect moves from a treated surface it has an opportunity to land on an untreated surface the value of maximum surface coverage is evident. Field tests (McCauley, *et. al.*, 1948) on the effectiveness of residual treatments to different proportions of the available resting surface demonstrated that the complete coverage treatment was markedly

superior to varying degrees of partial treatment especially as the deposits became older.

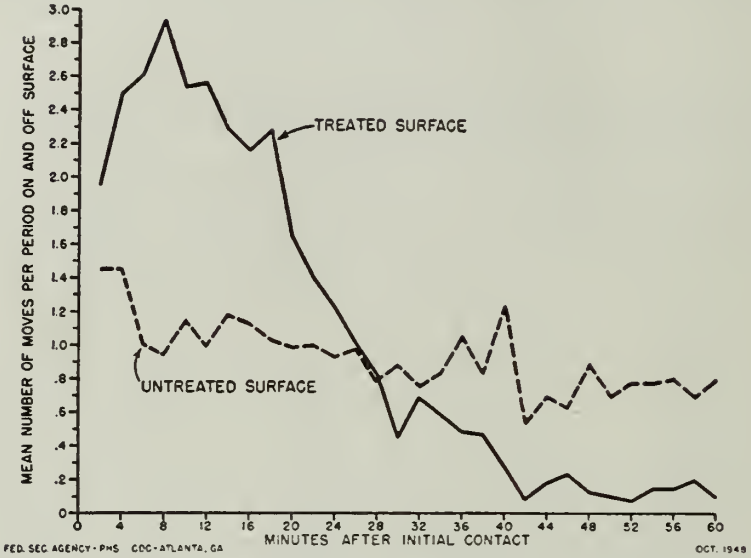

FIGURE 4. Mean number of moves on and off of untreated and DDT-treated surfaces shown by adult female *A. quadrimaculatus* mosquitoes at 2-minute intervals after the initial contact.

TABLE 2

Range and mean number of changes off and onto DDT-treated surfaces by adult female A. quadrimaculatus mosquitoes at definite time intervals after the initial contact with the test surface

TEST SURFACE	TREATMENT MG. DDT PER SQUARE FOOT	TIME INTERVAL AFTER 1ST CONTACT	NUMBER OF SPECIMENS	NUMBER OF CHANGES		
				Range	Mean	Standard error
Filter paper	200	0– 2	70	0– 8	1.85	0.27
		0–10	70	0–32	13.63	1.04
		0–20	70	2–57	26.8	1.56
		20–40	45	0–22	7.2	0.95
		40–60	20	0– 8	1.2	0.47
Plywood	200	0– 2	70	0–11	2.08	0.29
		0–10	70	0–29	10.93	0.84
		0–20	70	3–43	20.7	1.13
		20–40	45	0–20	8.9	0.78
		40–60	20	0–11	1.55	0.62

In the individual studies, the activities of the mosquitoes were recorded at 5-second intervals during the total observation time with differentiation into categories of still, walking, and flying periods. If a test insect probed the test surface with the proboscis or cleaned her antennae, legs, or wings during any 2-minute period after the initial contact this fact was noted in each period but not the duration of the activity.

The activity pattern of the adult female mosquitoes exposed to untreated surfaces of plywood and filter paper (figure 5) was quite uniform over the 1-hour observa-

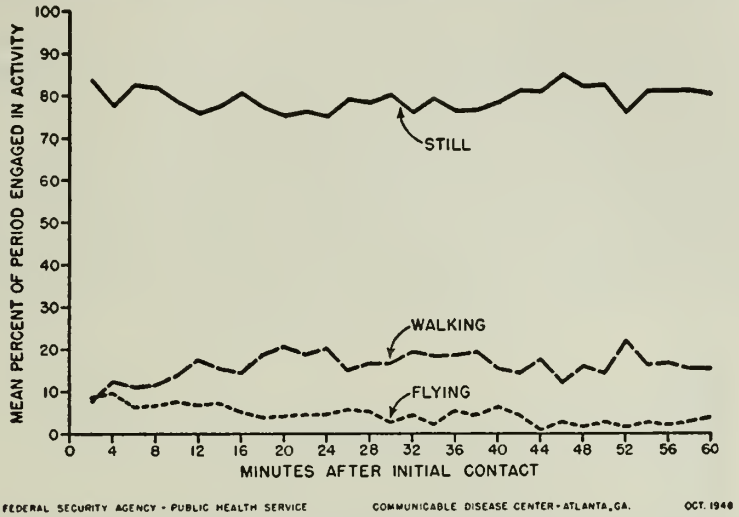

FIGURE 5. Mean percent of each 2-minute period after the initial contact with untreated test surfaces spent still, walking, and flying by adult female *A. quadrimaculatus* mosquitoes.

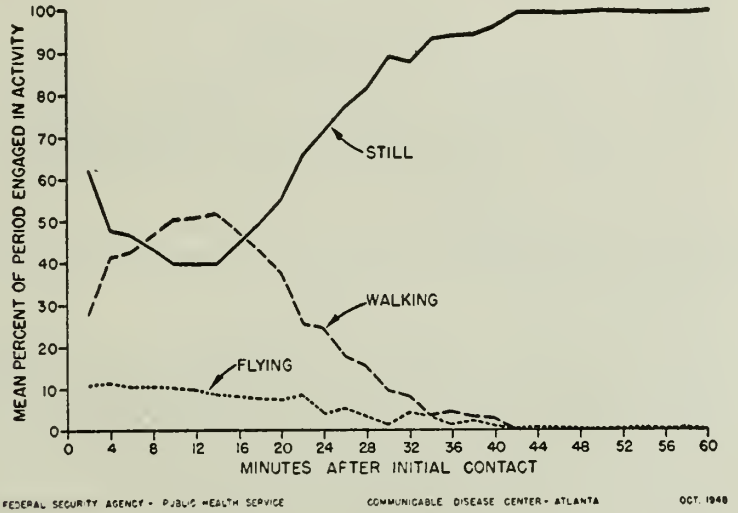

FIGURE 6. Mean percent of each 2-minute period after the initial contact with DDT-treated test surfaces spent still, walking, and flying by adult female *A. quadrimaculatus* mosquitoes.

tion time with the insects still 80 per cent of the time, walking 16 per cent of the time, and flying 4 per cent of the time. The activity pattern of insects exposed to DDT-treated surfaces, however, (figure 6) showed marked differences from the normal behavior pattern. During the first 2-minute period after the initial contact with DDT the insects had been excited so that they were still only 62 per cent,

walked 28 per cent, and flew 10 per cent of the time. The excitant effects increased during the first 14 minutes after the initial contact until at this time the insects were still about 40 per cent, walked about 50 per cent, and flew 10 per cent of the time. For the remainder of the observation time the insects were still an increasing proportion of each successive 2-minute period. The mosquitoes were not observed walking longer than 42 minutes after the initial contact with DDT but erratic spurts of flying activity occurred to the end of the 1-hour observation period.

Mosquitoes observed on untreated plywood and filter-paper surfaces during the first 2 minutes after the initial contact (figure 7) did not probe the surfaces, but within 10 minutes after the first contact and for the duration of the 1-hour observation period an average of 15 per cent of the mosquitoes were seen to probe the surfaces at least once during the successive 2-minute periods. Body-cleaning opera-

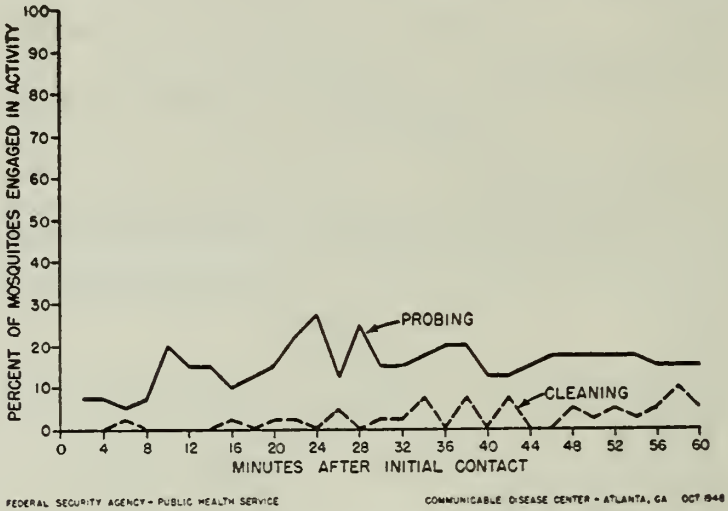

FIGURE 7. Percent of adult female *A. quadrimaculatus* mosquitoes probing and cleaning at various 2-minute intervals after initial contact with untreated test surfaces.

tions were noted in an average of 5 per cent of the mosquitoes per 2-minute interval after the initial contact but were quite sporadic over the 1-hour period. On the DDT treated surfaces (figure 8) the percentage of the test insects showing probing reactions was increased more than twice the normal percentage during the first 34 minutes after the initial contact and did not decline to the normal percentage until 46 minutes after contact. Cleaning activities were evidenced by more than 20 per cent of the observed mosquitoes from 6 to 20 minutes after the initial exposure but dropped rapidly to cease entirely at 34 minutes after the initial contact.

Although the method used in these observations was designed to allow the mosquito to contact DDT normally, i.e., foot contact only, the probing and cleaning activities are probably a factor in distributing the DDT to other parts of the body.

The progressive toxic action of DDT on mosquitoes at 2-minute intervals over a 1-hour observation period (figure 9) showed the following symptomatic stages: agitation, ataxia, hind leg paralysis, mid leg paralysis, front leg paralysis, and complete prostration. The appearance of agitation was quite rapid and more than 50

per cent of the mosquitoes showed visible signs of agitation within 4 minutes after the initial contact with DDT-treated surfaces. Twenty per cent of all the mos-

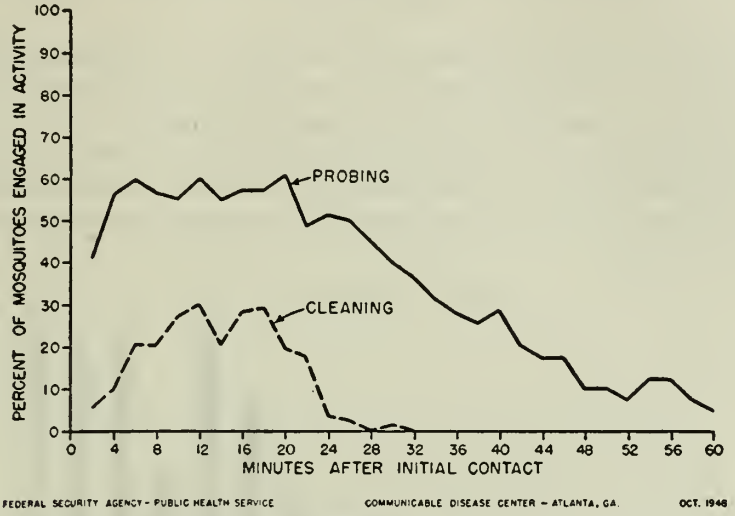

FIGURE 8. Percent of adult female *A. quadrimaculatus* mosquitoes probing and cleaning at various 2-minute intervals after initial contact with DDT-treated test surfaces.

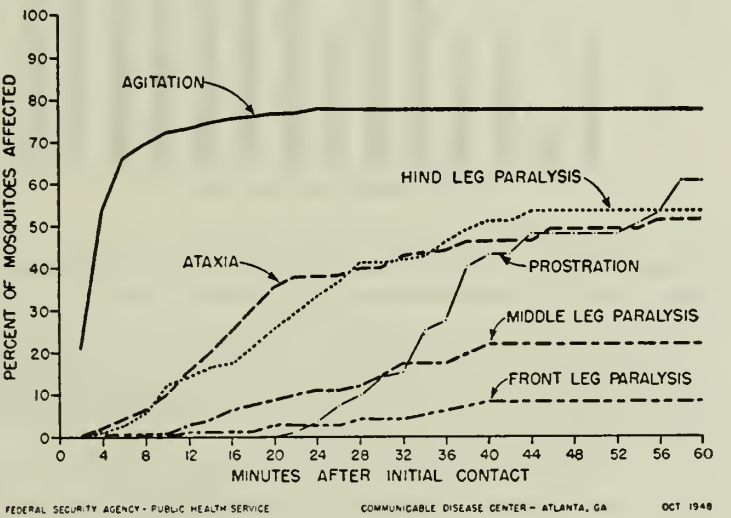

FIGURE 9. Percent of adult female *A. quadrimaculatus* mosquitoes showing various physiological manifestations of DDT toxicity at 2-minute intervals after the initial contact with DDT-treated surfaces.

quitoes observed failed to show definite signs of agitation at any time during the 1-hour observation period.

Ataxia was first evidenced by the failure of the insect to land on vertical surfaces properly and by the fact that more tarsal contacts with the surface were necessary to maintain a resting position. Paralysis of the hind legs was closely parallel in

extent and time of appearance to the ataxia. These symptoms initially appeared about 4 minutes after the initial contact with DDT. The number of insects showing this stage of DDT toxicity was slightly above 50 per cent at the end of the 1-hour observation period.

The paralysis of the legs progressed forward. The number of specimens in which paralysis of the middle and front legs was detected was about 20 per cent and 10 per cent respectively at the end of the 1-hour observation period.

Complete prostration first appeared at 22 minutes after an initial contact with DDT. The number of mosquitoes prostrate on their backs increased rapidly and at the end of the 1-hour observation period about 60 per cent of the mosquitoes were on their backs.

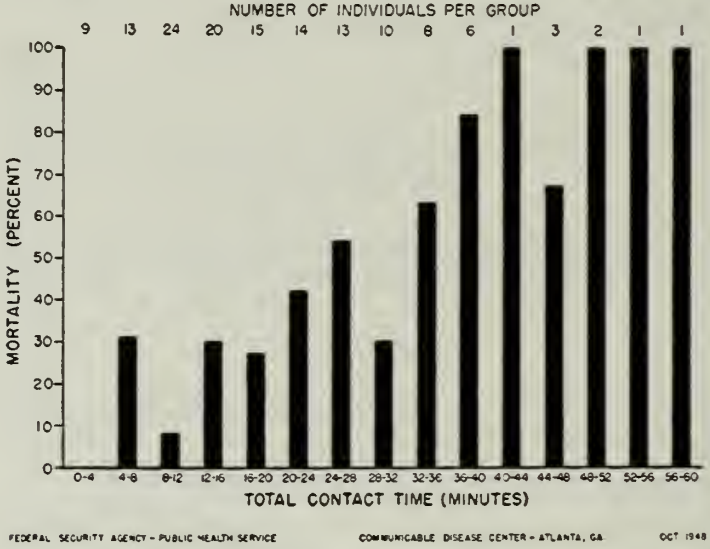

FIGURE 10. Twenty-four-hour mortality (percent) of adult female *A. quadrimaculatus* mosquitoes after actual contact with DDT-treated surfaces for definite lengths of time.

It has been frequently noted that in the later stages of DDT paralysis the adult insects will autotomize from one to five legs. This process is almost instantaneous and the autotomize legs have been seen to twitch convulsively for periods as long as 30 minutes after separation from the body.

The total contact period with the DDT-treated surface was recorded for each of the individual exposure studies. Since the mosquitoes could move on and off of the treated surface at will a wide range of total contact periods from 50 seconds to 58 minutes and 55 seconds on the treated surface was recorded. For comparative purposes the total contact periods falling within each 4-minute interval from 0 to 60 minutes were considered as separate groups and the mortality was calculated for each group. The mortalities have been based on observations taken 24 hours after the exposure period. Checks were made on the mortality at 48, 72, and 96 hours after tests but relatively little additional mortality was encountered after 24 hours. These observations (figure 10) indicate that an actual contact with DDT of 20 to 32 minutes produced 50 per cent mortality of adult female *A. quadrimaculatus* under the test

conditions. Actual contact of more than 40 minutes was required for 90 per cent mortality.

DISCUSSION

Considering all observations regardless of contact time, only 49 of the 140 mosquitoes (35 per cent) were dead at the end of the holding period. Eighty-four individuals (60 per cent), however, were observed to be prostrate on their backs at the end of the 1-hour observation period. The correlation of these two facts indicates that adult mosquitoes may show all signs of DDT toxicity up to complete prostration and still not succumb to the lethal effects of DDT. These data confirm the work of Kennedy (1947) and also clarify a previous assumption that most mosquitoes once knocked down are unable to recover. Many of the prostrate insects suffered the loss of one to three legs through autotomization and yet were still capable of flying.

Since 20 per cent of the test insects failed to show any sign of DDT agitation it is possible that some of the population escaped the lethal effects of contact with deposits of 200 mg. DDT per square foot either (a) by short exposure periods from chance resting on untreated surfaces in the exposure chamber, or as was indicated by several examples, (b) the possibility of resting on DDT-treated surfaces without absorbing or possibly not contacting any DDT.

In view of the fact DDT excites the insects so that they leave treated surfaces several times before a lethal contact period has been attained, the probability of escape is increased. Therefore the effectiveness of DDT applications in the demonstrated control of malaria may well be due largely to the interruption of parasite transmission from insect to man in a sufficient degree to reduce the incidence of the disease.

SUMMARY

Individual observations over periods of 20, 40, and 60 minutes from the time of initial contact with either an untreated or DDT-treated surface of plywood or filter paper have been made on 180 adult female *A. quadrimaculatus* mosquitoes. Each female was confined in an observation chamber with free access to both DDT-treated and untreated resting surfaces. The observation period was started at the time of initial contact with the test surface. The number of points of contact, the general activity, the number and duration of the contacts with the test surface, the signs of DDT toxicity, and the mortality at the end of a 96-hour holding period were recorded.

The preference of the insects for the plywood and filter-paper test surfaces over the glass and plexiglass surfaces of the remainder of the chamber is shown by the fact that they remained in contact with untreated test surfaces about 75 per cent of the observation period. As an average, they moved on and off the surface once every 2 minutes. They were still 80 per cent, walked 16 per cent, and flew 4 per cent of each 2-minute interval after the initial contact over the entire 1-hour observation period. During any 2-minute interval after the initial contact, about 15 per cent of the mosquitoes was seen to probe the untreated test surface at least once and 5 per cent demonstrated body-cleaning operations. The normal pattern of ac-

tivity of the mosquitoes did not show any marked modifications during the 1-hour period on the untreated surfaces.

During the first 20 minutes after the initial contact with test surfaces treated with 200 mg. DDT per square foot, the test insects were stimulated to move on and off the surface about 2.5 times per 2-minute period, were in contact abut 40 per cent of each 2-minute interval and engaged in more walking, flying, probing, and cleaning operations than normal. Signs of agitation, ataxia and leg paralysis were noted.

From 20 to 40 minutes after the initial contact with the treated surface, the mosquitoes were noted to cling to resting surfaces regardless of texture, moved on and off the surface 1.0 time per 2-minute period, were in contact about 60 per cent of each 2-minute interval and engaged in decreasing amounts of walking, flying, and probing. Body-cleaning operations essentially stopped. No additional agitation was noted, but the paralytic effects were more severe and complete prostration increased rapidly.

From 40 to 60 minutes after the initial contact with the treated surface, the adult females moved on and off the surface less than 0.2 time per 2-minute interval, were in contact with the test panel about 30 per cent of the time, and exhibited only occasional short sporadic flights. Walking activity had virtually ceased and probing was greatly decreased. Little additional paralysis was noted but the proportion of insects showing complete prostration continued to increase.

In contrast to other anopheline species noted in the literature, actual contacts with DDT deposits for periods of 20 to 28 minutes and of at least 40 minutes were necessary to produce mortalities of 50 and 100 per cent respectively with *A. quadrimaculatus* adult females.

REFERENCES

Elmendorf, J. E., Jr., Marucci, P. E., Griffin, J. B., Meyer, S. L., and Ryan, G. S.: Longevity of killing effect of DDT for mosquitoes contacting screen wire painted with DDT solutions. Amer. J. Trop. Med., **26** (5): 663–686 (1946).

Fay, R. W., Simmons, S. W., and Clapp, J. M.: Laboratory investigations on the toxicity of DDT residues to adults of *Anopheles quadrimaculatus*. Supp. No. 186, Pub. Health Rep., 21–34 (1945).

Hocking, K. S.: The residual action of DDT against *A. gambiae* and *A. funestus*. Trans. Roy. Soc. Trop. Med. and Hyg., **40** (5): 589–601 (1947).

Kartman, L., and da Silveira, M. M.: Effect of short contact with DDT residues on *Anopheles gambiae*. J. Econ. Ent., **39** (3): 356–359 (1946).

Kennedy, J. S.: The excitant and repellent effects on mosquitoes of sub-lethal contacts with DDT. Bull. Ent. Res., **37** (4): 593–607 (1947).

Knipling, E. F.: The development and use of DDT for the control of mosquitoes. J. Nat. Mal. Soc., **4** (2): 77–92 (1945).

MacInnes, D. G.: On the effect of DDT-treated surfaces on adults of *Anopheles gambiae* and *A. funestus*. Bull. Ent. Res., **38** (1): 123–130 (1947).

McCauley, R. H., Fay, R. W., and Simmons, S. W.: The importance of coverage in DDT residual house spraying for control of *Anopheles quadrimaculatus* mosquitoes. Pub. Health Rep., **63** (13): 401–407 (1948).

Metcalf, R. L., Hess, A. D., Smith, G. E., Jeffery, G. M., and Ludwig, G. W.: Observations on the use of DDT for the control of *Anopheles quadrimaculatus*. Pub. Health Rep., **60** (27): 753–774 (1945).

Thompson, R. C. M.: The effects of house spraying with pyrethrum and with DDT on *Anopheles gambiae* and *A. melas* in West Africa. Bull. Ent. Res., **38** (3): 449–464 (1947).

SEASONAL HISTORY OF *ANOPHELES QUADRIMACULATUS* IN THE TENNESSEE VALLEY

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INTRODUCTION

During the fifteen years in which the Authority has been carrying out a malaria control program on its impoundments in the Tennessee Valley, routine inspections for *Anopheles quadrimaculatus* have been used to provide indices of the effectiveness of anti-mosquito measures. During this same period, studies have been made on overwintering and other aspects of the seasonal history of this mosquito. As a result, there has been accumulated a considerable amount of data, only a portion of which has previously been published. The purpose of the present paper is to make a general summary of the seasonal history of *A. quadrimaculatus* in the Tennessee Valley based on the observations of the past fifteen years and to present some of the recent information on overwintering habits and seasonal abundance.

In addition to the individuals listed in the references, almost every member of the Biology Staff has made some contribution to our knowledge of the seasonal history of *A. quadrimaculatus*. Acknowledgment is also made to Miss Helen T. Chandler and Miss Caroline E. Wilson for tabulating most of the data on seasonal abundance.

OVERWINTERING

Hinman and Hurlbut (1940) discussed the winter activities and hibernation of *A. quadrimaculatus* in the Tennessee Valley and reviewed the literature on this subject. Considerable additional data subsequently have been accumulated. Observations on hibernating populations of *A. quadrimaculatus* in caves have been continued so that this aspect of the seasonal history of this mosquito can now be rather clearly defined. In October when the adult females are disappearing from barns and other summer diurnal resting places, they begin to appear in caves (Fig. 1). The number of these hibernating adults increases rapidly through November and reaches a peak near the end of the month. Through the remainder of the winter cave populations of hibernating adults steadily decrease and only an occasional specimen may be found beyond the middle of February. Temperature appears to be the nost important factor governing the entrance and departure of the hibernating adults from the caves. It will be noted from Fig. 1 that the sharp increase in the numbers of hibernating adults in November coincided with a period when the noon day temperatures outside the cave reached the level of those inside the cave; likewise, the exodus of the hibernating adults during January and February coincided with periods when the outside temperatures again reached the level of those inside the cave. The steady decrease

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in cave population from late November through December is probably due to a natural dying off of the mosquitoes such as has been experienced in the artificial cave of the outdoor insectary at Wilson Dam, and mortality in the natural caves may also be increased by predators.

It is interesting to note that even a short distance back in an open cave the temperatures never reach freezing although the outside temperatures may fall far below freezing. For example, in early February of 1945 when the temperature outside the Cave Springs Cave on Wheeler Reservoir dropped to 10°F. the temperature inside the cave where *A. quadrimaculatus* adults were hibernating only reached 35°F.

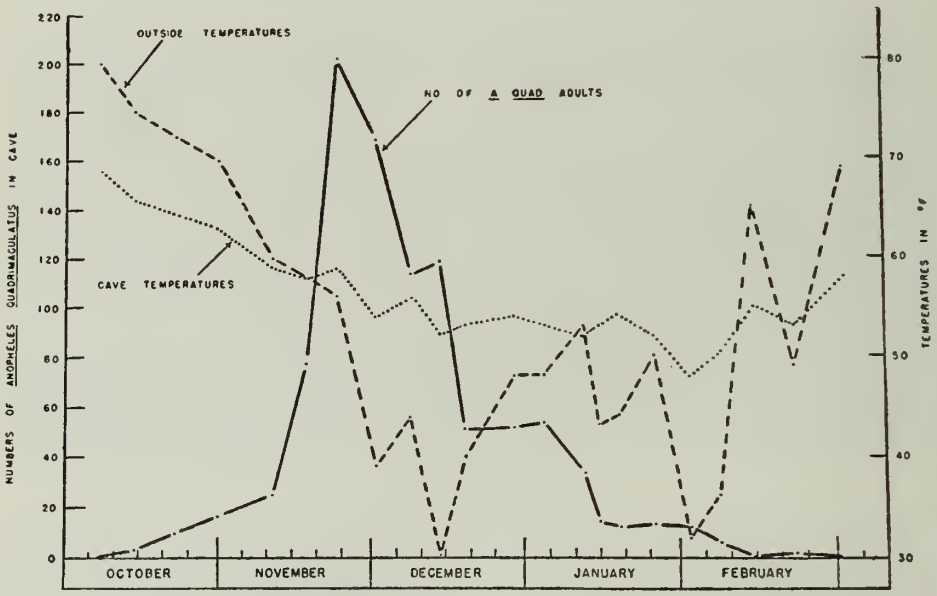

FIG. 1. Populations of *A. quadrimaculatus* and indoor and outdoor temperatures in the Cave Springs Cave, Wheeler Reservoir, 1944-45 (temperatures recorded between 11 a.m. and 1 p.m.).

It has been previously reported (Ives, 1938) that overwintering adults concentrate in the twilight zone. Although this has generally been true in the studies in the Tennessee Valley, other factors appear to play an important role. For example, on cold windy days the mosquitoes appeared to move farther back into the cave and many would be found in the darkest portions. At other times when the ceiling of the twilight portion of the cave became wet from condensation, the mosquitoes were found to have moved back into the darker portions of the cave where the ceiling was dry. The humidity in the caves was relatively constant, varying between 76 and 100 when the outside range was 35 to 100.

The high humidity and low temperatures in the hibernation caves apparently provide very favorable conditions for the survival of the adult mosquitoes. Hibernating adults collected from caves and transported to an artificial cave at Wilson Dam where temperature and humidity conditions were comparable survived for as long as 133 days. Since they were probably at least a week or two old when they went into hiber-

nation, this would appear that the life span of hibernating *A. quadrimaculatus* adults may be as long as five months. Hinman and Hurlbut (1940) also reported a survival of reared specimens of *A. quadrimaculatus* in an outdoor insectary for at least 133 days. This extended longevity of the overwintering adults is in great contrast to the relatively short life span of the summer adults. Hurlbut (1941) reported that female *A. quadrimaculatus* adults reared out of doors under semi-natural conditions during the middle of the summer had an average survival time of only about 16 days with a maximum survival of 31 days.

Of particular interest during recent years has been the demonstration that, at the latitude of North Alabama, *A. quadrimaculatus* may emerge from hibernation during periods of warm weather at any time during the winter to take blood meals. In the Wilson Dam area outdoor biting records for *A. quadrimaculatus* have been obtained during every month of the year. Apparently the species exhibits a true gonotrophic dissociation at this latitude since adults which take blood meals during the middle of winter return to hibernation without developing eggs. There is, however, a considerable drop in blood-feeding activity during November and December, and it is frequently impossible to get hibernating adults which have been collected from caves and brought into the insectary to take blood meals. Hinman and Hurlbut (1940) in their observations during the winter of 1938–39 reported that no specimens were found in nature with blood in their stomachs between October 25 and the first part of February. However, Boyd and Weathersbee (1929) found occasional blooded specimens of *A. quadrimaculatus* in winter collections at 36°N. latitude in coastal North Carolina where the average temperature is only slightly above that at Wilson Dam. Most of the outdoor winter blood-feeding records in the Wilson Dam area have been obtained when the air temperatures were between 65° and 75°F., although feeding has occurred at temperatures as low as 52°F. During winters characterized by sustained periods of cold weather there may be very little blood-feeding activity. For example, during the winter of 1945–46 no blood-feeding records were obtained during the three-month period from November 5 to February 4.

Fat body development reaches a peak in the late fall and then diminishes through the winter. There is little evidence of ovarian development until about the time when the adults emerge from hibernation in early February. These adults take blood meals and deposit eggs which produce the first new brood of adults for the season. The development of this first brood is greatly retarded by the low temperatures which prevail at this time of the year. Observations in the outdoor insectary at Wilson Dam indicate that overwintering adults which take their last blood meals in early February require a period of 2 to 3 weeks or more to complete the development of the ovaries so that the first eggs are not deposited until the last part of February or the first part of March. These eggs require one to two weeks to hatch and the larvae develop very slowly so that pupation does not take place until about the middle of April. The pupal period lasts about a week and the first brood of adults generally emerges during the last half of April, though a few adults may be found during the first half of April. Under outdoor conditions the minimum time which has been observed for development of the first brood from egg to adult has been about 50 days with a maximum of about 70 days so that it appears that an average period of about

two months is required to complete the life cycle at this season of the year. This is in contrast to the development rates reported by Keener (1945) for insectary conditions (76–80°F.) where the period from egg to adult averaged only three weeks with a minimum of two weeks and a maximum of about four weeks. Hurlbut (1943) found that insectary-reared *A. quadrimaculatus* would complete development from egg to adult under outdoor summer temperatures at Wilson Dam, Alabama, in as short a period as 12 days, but that during the early spring (April) this development might require as much as 38 days.

Field studies and outdoor insectary studies at Wilson Dam have provided no evidence that *A. quadrimaculatus* may survive the winter in the larval stage at this latitude, and it appears that hibernating inseminated female adults carry the species from one season until the next. The latest record of survival for larvae kept under semi-natural conditions in a large tank in the outdoor insectary has been December 5 and the latest collection of *A. quadrimaculatus* larvae in the field was also during the first week in December. Larvae have not been collected in the field between that date and the last part of April. The small size and scarcity of larvae during March and early April probably explain the failure to collect them at that time.

A considerable amount of information has been obtained on the relation of water surface temperatures to the spring occurrence and development of *A. quadrimaculatus* larvae. Approximately 2,000 spring collections of larvae were made in which records were kept of the water surface temperatures at the time and place of collection. An analysis of these records shows that *A. quadrimaculatus* larvae generally did not appear in significant numbers until the afternoon water surface temperatures reached 65–75°F. and that this species did not become dominant in collections until the temperatures reached 90–95°F. Although the larvae can withstand low temperatures and have even been observed to survive several hours of being frozen in solid ice, development is very slow at temperatures below 55°F. Optimum development appears to take place when the water temperature is between 85 and 95°F. although the larvae will survive, at least for short periods, when the temperature is as high as 105°F. The effect of temperatures during February and March on the development of the first brood will be discussed in the next section.

SEASONAL ABUNDANCE

A detailed analysis has been made of data on seasonal population densities of *A. quadrimaculatus* which the Authority has accumulated during the past fifteen years of routine mosquito inspection services. This information includes 52 reservoir years[2] of inspection on the 5 lower main river reservoirs, 33 reservoir years of inspection on the 4 upper main river reservoirs, and 49 reservoir years of inspection on the storage reservoirs, making a total of 134 reservoir years of inspection involving approximately 200,000 routine collections from catching stations.

A study of meteorological data from the Tennessee Valley area has revealed that the grouping of the reservoirs by upper and lower main river regions coincides rather closely with the natural temperature divisions between endemic and non-endemic

[2] A reservoir year of inspection is defined as the carrying out of routine inspections around one reservoir for one full mosquito breeding season.

malaria areas. The 60° isotherm which is generally adopted as the dividing line between these areas is usually shown as cutting across the Tennessee region in the middle portion of the Kentucky Reservoir area. However, it has been found that Paducah, Kentucky, has an annual mean temperature of 60°F. and it appears that the entire Kentucky Reservoir area therefore lies below the 60° isotherm which roughly parallels the Valley across northern Alabama and crosses it somewhere in the vicinity of Hales Bar Reservoir (Fig. 2).

The average densities of *A. quadrimaculatus* per station per week for the three reservoir groups during the past fifteen years are shown in Fig. 3. It will be noted that on the lower main river reservoirs significant production usually begins during the first part of May. Bradley and Fritz (1945) have reported that in the 60–65° isothermal zone significant *A. quadrimaculatus* production begins during the first part of April and that in the 55–60° zone it begins during the last part of May. The lower main river reservoirs of the Tennessee Valley approximately parallel the 60° isotherm, which is the boundary between the above two zones. The initiation of significant production in these reservoirs during the first part of May is therefore in accordance with the findings of the above workers since this is just about half way between the dates which they reported for the two adjacent zones. Significant production on the upper main river reservoirs does not begin until the first part of June which is somewhat later than the last of May which is the time reported by Bradley and Fritz for the beginning of significant production in the 55–60° zone in which these reservoirs lie.

Fig. 3 clearly illustrates the higher densities and more extended breeding period in the lower main river reservoirs as compared with the upper main river and storage reservoirs. Whereas peak productions in the lower main river reservoirs usually occur from the middle of June until the middle of August, the peak on the upper main river reservoirs usually occurs between the middle of July and the end of August. Peak production on storage reservoirs does not generally take place until the latter part of August or September. This late peak is undoubtedly due to the fact that significant production usually does not take place in the storage reservoirs except during abnormal years when the water has been low during the spring and summer growing season and then rises into the marginal vegetation during the late summer and fall. The progressive seasonal lag in the emergence of *A. quadrimaculatus* in the three reservoir groups is of real practical significance since it makes possible more widespread usage of mobile larviciding equipment such as airplanes.

The population curves given in Fig. 3 are based on actual quantitative data and therefore are strongly weighted by years of unusually heavy production such as was experienced in 1941 when during the first week in August a single station (120) on Wheeler Reservoir had an estimated count of 10,000 adult *A. quadrimaculatus*. For this reason, a different type of analysis has been made in which the average counts for each week of the inspection season are expressed as the percent of the maximum count for the season. In this way, the peak production for each year is given equal weight and when the results are averaged together a more accurate picture is given of the time when peak productions may be expected regardless of the actual magnitudes of the peaks. Seasonal curves for the upper and lower main river reservoirs expressed

FIG. 2. Outline of the Tennessee Valley area showing the approximate location of the 60° isotherm which is generally accepted as the boundary between endemic and non-endemic malaria areas.

in such per cents of maximum are given in Fig. 4. These curves demonstrate clearly the lag of approximately one month between the upper and lower reservoir areas. Whereas average counts usually reach 10 per cent of maximum on the lower main

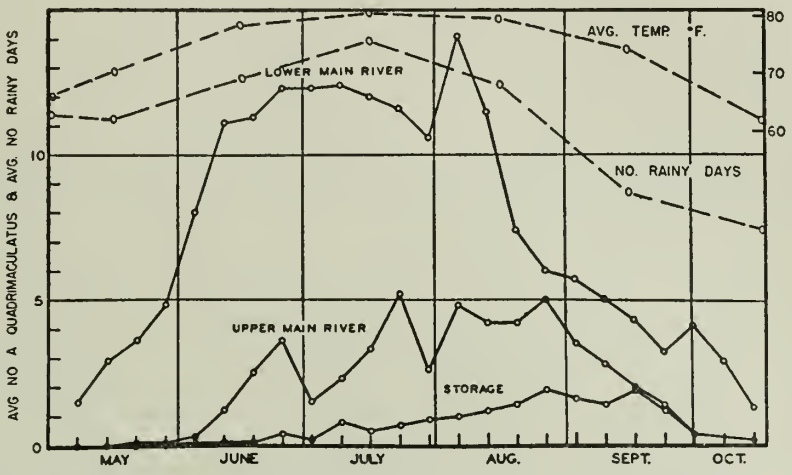

FIG. 3. Average densities of *A. quadrimaculatus* per station per week for the three reservoir areas of the Tennessee Valley as determined by routine inspections during a 15-year period from 1934–1948, nclusive.

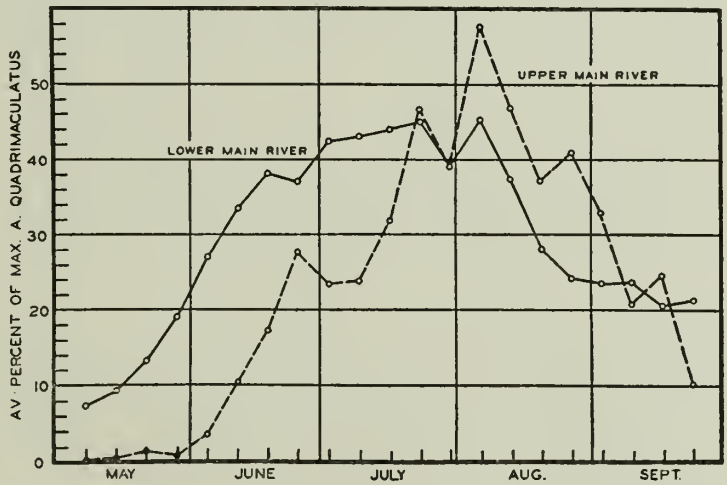

FIG. 4. Seasonal population curves for *A. quadrimaculatus* in the upper and lower main river reservoirs expressed as average per cent of maximum.

river reservoirs by the middle of May, this degree of production does not take place on the upper main river reservoirs until almost the middle of June. Furthermore, although the lower main river reservoirs show an extended period during July and the first part of August when maximum densities may take place, the period when maximum counts may be expected on upper main river reservoirs is much more nar-

rowly limited, usually occurring during the first half of August. Both the per cent of maximum and the actual quantitative curves show definite indications of brood peaks, although these are partially obliterated by coalescence due to seasonal variations.

Peak productions of *A. quadrimaculatus* coincide with the period of maximum summer temperatures, but average temperatures during the fall when mosquito densities are decreasing are actually higher than in the spring when populations are building up (Fig. 3). Thus, it would appear that summer temperatures are not a limiting factor with reference to seasonal population densities. The three months when mosquito counts are the highest also show the highest average number of rainy days, and the fall drop-off in anophelism coincides with a sharp decline in the number of rainy days. This would suggest that decreased mosquito production during the latter part of the season is due primarily to a reduction in aquatic habitats suitable for *A. quadrimaculatus* production rather than to lowered temperatures. The frequent occurrence of peak productions in the storage reservoirs late in the season and the tremendous emergence of *A. quadrimaculatus* which resulted from the initial impoundage of Kentucky Reservoir in the fall of 1944 further confirm this relationship. In the latter instance mosquito densities actually reached a peak during the second week in October and dropped off only when minimum temperatures approached the freezing point. It would therefore appear that the drop in mosquito production on the main river reservoirs during August and September is due primarily to the elimination of favorable breeding areas by water level recession rather than to unfavorable temperature conditions. With this in mind, a comparison has been made between the average seasonal population curve for the lower main river reservoirs and the Buckeye-Blackwell Swamp area of Wheeler Reservoir, a region in which swampy conditions and limited drainage have maintained a favorable aquatic habitat for *A. quadrimaculatus* when most areas of production have been eliminated by water level recession. A comparison of these two curves expressed in average per cents of maximum is given in Fig. 5. It will be observed that through July the curves are very similar but that during the first part of August counts in the Buckeye-Blackwell Swamp area continue to build up to a maximum when counts from other reservoir areas are dropping off. Thus, it appears that the seasonal population curves in the reservoir areas are strongly influenced by the water level recession schedules and are not characteristic of natural breeding areas, particularly during the latter part of the season. This is in agreement with the findings of Bradley and Fritz (1945) who reported peak densities of *A. quadrimaculatus* occurring through September in the 60–65°F. isothermal zone.

In studying the mosquito density records for the various years, it seemed apparent that factors other than water levels were influencing the seasonal magnitude of mosquito productions. It was felt that temperatures or humidity during the normal mosquito breeding season might influence average production rates from year to year. However, a general study of these factors gave no indication of any such correlation. For example, during 1945, the year of maximum production on the lower main river reservoirs, and 1947, the year of minimum production, conditions were remarkably similar. This is evidenced by the following summary for the period of April through

July which covers the breeding season from the beginning through the peak of production for these two years.

YEAR	TOTAL PRECIPITATION	AVERAGE TEMP.	AVG. RELATIVE HUMIDITY (DURING DAY)	AVERAGE % SUNSHINE	NUMBER OF CLOUDY DAYS
1945	15 inches	72° F.	66	55	37
1947	15 inches	72° F.	66	51	37

The uniformity of mosquito breeding conditions in the reservoirs from year to year is also increased by standardized water level management schedules which result in the creation of breeding areas of similar extent during comparable periods of the

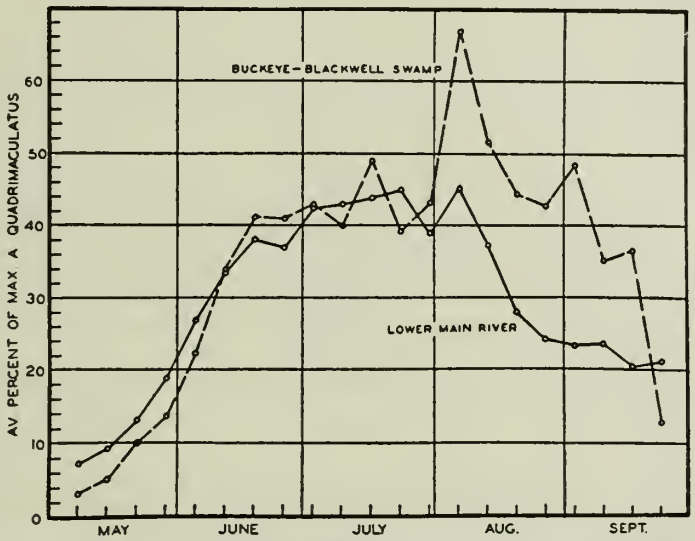

FIG. 5. Seasonal population curves for *A. quadrimaculatus* in the Buckeye-Blackwell Swamp Area of Wheeler Reservoir and the lower main river reservoirs expressed in average per cent of maximum.

breeding season. It, therefore, occurred to us that perhaps conditions during the earlier critical period of February and March when mosquitoes are emerging from hibernation to oviposit and the first brood of larvae is developing might influence production during the following breeding season. Accordingly, a comparison was made between the year of highest production (1945) and the year of lowest production (1947) on the lower main river reservoirs with reference to conditions during February and March. It will be observed (Fig. 6) that during both of these years average temperatures were quite comparable and close to normal during the May to September mosquito breeding period. However, in 1945 temperatures were much above normal in February and March, whereas in 1947 the February-March temperatures were much below normal. Following this clue, a correlation was made between average mosquito densities and temperatures for February and March for each of the past fifteen years during which routine mosquito inspections have been carried out on the lower main river reservoirs. The results (Fig. 7) show that during nine of the

fifteen years there was a very close correlation between average mosquito densities and February-March temperatures and that there was a reasonable conformity

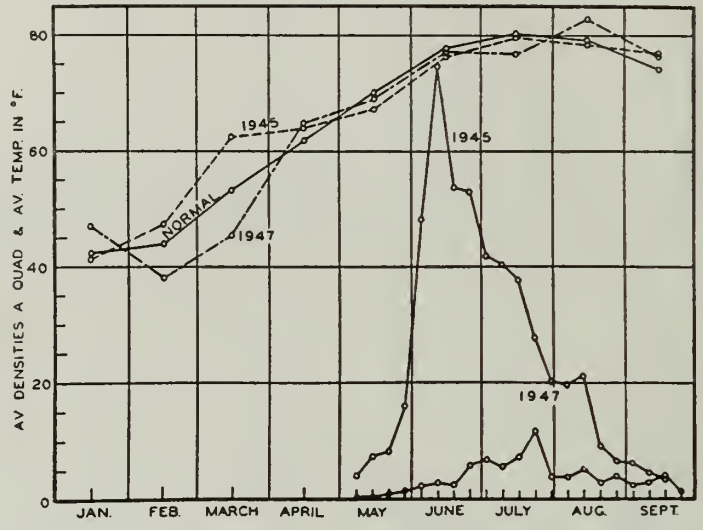

FIG. 6. A comparison of *A. quadrimaculatus* populations and average monthly temperatures in the lower main river reservoir area of the Tennessee Valley during 1945 and 1947 which were the years of the highest and lowest production, respectively, in the history of the TVA impoundments.

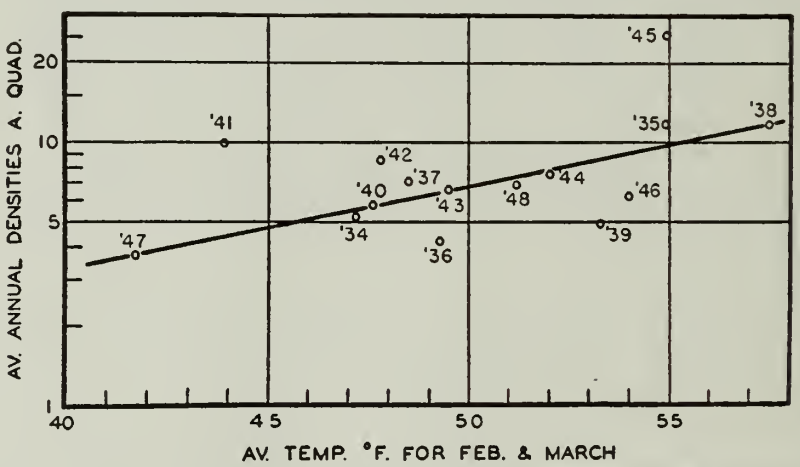

FIG. 7. The relation between the average annual densities of *A. quadrimaculatus* per station per week and the average temperatures during February and March in the lower main river reservoir area of the Tennessee Valley during the period of 1934 to 1948, inclusive.

during four of the remaining six years. In only two of the fifteen years (1941 and 1945) did the data fall far out of bounds. In 1941, water levels on the main river reservoirs were held down during the first part of the growing season and then brought back up into the heavy marginal growth which had invaded the zone of fluctuation.

This provides ample explanation for the occurrence of much heavier production in 1941 than would have been expected from the correlation curve. In 1945 the highest production in the history of the TVA impoundments occurred. This year not only had temperatures during February and March which were far above normal but the unusually warm weather continued through April and very favorable conditions for production prevailed during the subsequent two months, thus resulting in an unusually heavy production.

It seems apparent from these studies that under normal conditions of water level management the extent of production of *A. quadrimaculatus* will be directly correlated with temperatures which occur during February and March. High temperatures during this period result in an increased emergence of the first brood of adults in April and by geometrical progression this brings an increasingly heavy production of adults from subsequent broods. Conversely, low temperatures during February and March limit the first brood resulting in much lower counts during the following mosquito breeding season. This basic correlation should be most helpful in predicting the extent of production of *A. quadrimaculatus* each season. At the end of March the temperatures for the past two months can be averaged and compared with the normal. A forecast can then be made a full month ahead of the normal mosquito breeding season as to what degree of production is to be expected. It will be interesting to test the application of this theory during subsequent mosquito breeding seasons.

SUMMARY

A summary is made of observations on the seasonal history of *A. quadrimaculatus* which have been made in the Tennessee Valley during the past fifteen years. In the region of North Alabama, this mosquito passes the winter as inseminated adult females which hibernate in caves and similar resting places. The mosquitoes begin to appear in the caves during October, reach a peak in late November, and disappear by the first part of February. Adults may emerge during periods of warm weather at any time of the winter to take blood meals but do not develop eggs until they leave the caves in early February. The first eggs are deposited during the latter part of February or the first part of March and the first brood of adults emerges during April. The first occurrence of adults in significant numbers in inspection stations usually coincides with the emergence of the second brood of adults. Peak productions occur from the middle of June until the middle of August, and adult densities usually reach insignificant numbers by the end of September.

Temperatures during February and March are shown to have an important effect upon the extent of mosquito production during the subsequent breeding season. This is a critical period in the seasonal history of the species and above-normal temperatures favor heavy production, whereas below-normal temperatures result in light production during the following season. This relationship may make it possible to forecast the degree of production to be expected a month ahead of the period when control operations are initiated.

The fall decline in adult densities of *A. quadrimaculatus* appears to be due to a decrease in extent of aquatic habitat suitable for mosquito breeding rather than to unfavorable temperatures.

REFERENCES

Boyd, M. F., and Weathersbee, A. A., 1929. Studies on the bionomics of North American anophelines. V. Winter activities of anopheline imagines in coastal North Carolina (36° N. Lat.). Amer. Jour. Hyg., Vol. 9, 682–694.

Bradley, G. H., and Fritz, Roy F., 1945. Observations on seasonal occurrence and abundance of *Anopheles quadrimaculatus* Say. Jour. Nat. Mal. Soc., Vol. 4, No. 3, 251–262.

Hinman, E. Harold, and Hurlbut, H. S., 1940. A study of winter activities and hibernation of *Anopheles quadrimaculatus* in the Tennessee Valley. Amer. Jour. of Trop. Med., Vol. 20, No. 3, 431–446.

Hurlbut, H. S., 1941. The longevity of adult female *Anopheles quadrimaculatus* in relation to malaria transmission with special reference to temperature and humidity. Unpublished TVA Report submitted to Health and Safety Department, Oct.

Hurlbut, H. S., 1943. The rate of growth of *Anopheles quadrimaculatus* in relation to temperature. Jour. of Parasitology, Vol. 29, No. 2, 107–113.

Ives, J. D., 1938. Cave hibernation of mosquitoes. Jour. Tenn. Academy of Science, Vol. 13, p. 15–20.

Keener, George G., Jr., 1945. Detailed observations on the life history of *Anopheles quadrimaculatus*. Jour. Nat. Mal. Soc., Vol. 4, No. 3, p. 263–270.

OVERWINTERING OF *ANOPHELES CRUCIANS* WIED. IN SOUTH CAROLINA

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Studies which have been carried on cooperatively between the South Carolina State Board of Health and the Public Health Service in the vicinity of Manning, South Carolina, indicate that *Anopheles crucians* (*crucians*) passes the winter in a manner characteristically quite different from either *A. quadrimaculatus* or *A. punctipennis*. The South Carolina lowland areas near Manning are characterized by extensive boggy bays, numerous streams, and swamps where brownish, acid water provides favored larval habitats for *A. crucians*. Barber, Komp and Hayne, (1924), observed all three *Anopheles* breeding continuously through the winter in South Georgia and on the Gulf Coast, but published data on winter activities of *A. crucians* farther north are scanty. Boyd and Weathersbee (1929) noted that at Edenton in coastal North Carolina winter behavior patterns of *A. punctipennis* and *A. quadrimaculatus* differ strikingly. The female *A. quadrimaculatus*, in diapause, hibernated from December to February, "whereas *punctipennis* is in such a state of reproductive activity that it might be regarded in this latitude as a winter species." The Edenton findings have been confirmed in this area for both species and, for *A. quadrimaculatus*, by Hinman and Hurlbut (1940) in the Tennessee Valley. *A. crucians*, the predominant *Anopheles* in the South Carolina observation area, appears to pass the winter in a third fashion and, therefore, it seems desirable to record our three years field data on this phenomenon.

PHENOLOGY

Keeping in mind Aristotle's dictum about seeking only so much exactness as may be allowed by the nature of the subject matter, the seasonal occurrence of *crucians* stages in the study area is as follows:

(1) Larvae are present throughout the year;

(2) Smaller larvae are practically lacking in late February and early March;

(3) Larger larvae virtually disappear the latter half of November and reappear about the middle of December;

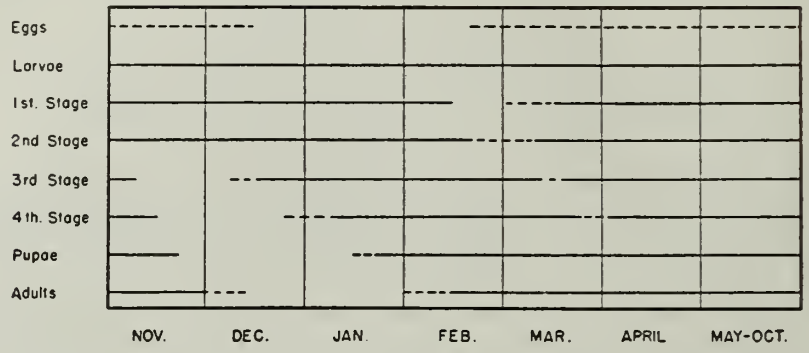
(4) Pupae disappear from mid-November until about mid-January;

(5) Adults are virtually absent from early December to early February;

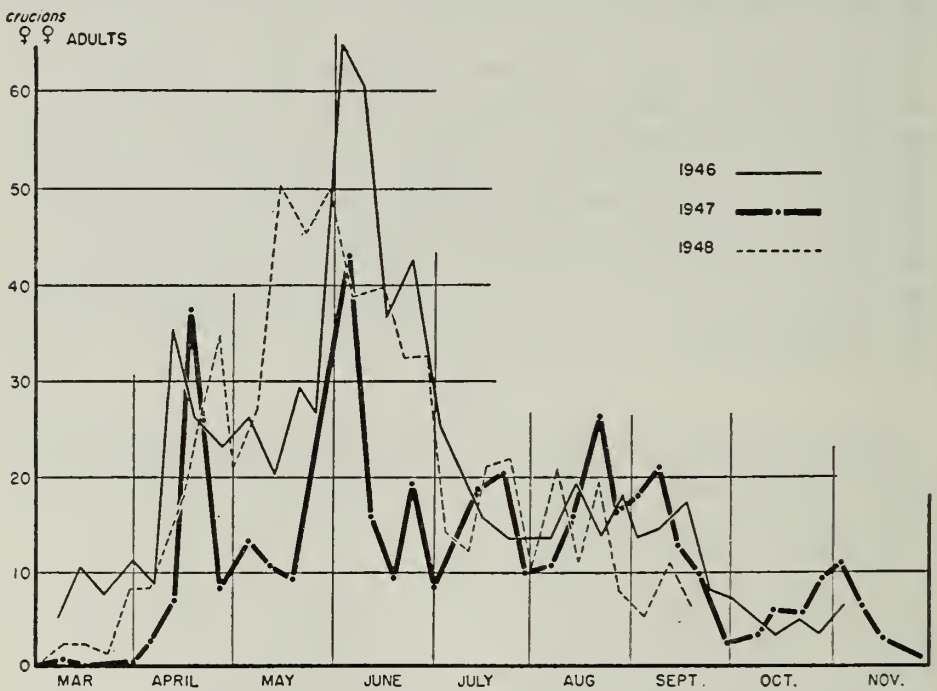
(6) Adults increase erratically from February through May, then decline more gradually in June to November.

These observations are based on adult counts and rearings from collections in which approximately 20,000 large larvae and pupae were taken. The results are summarized in Graphs I and II. Winter inspection data gained during warm periods are omitted because no adults were found in January. The first week in December, counts were less than 0.5 adult per station, and similar counts occurred the

last week or two of February. In Graph I data inadequately supported are indicated by dashes.



GRAPH I. *A. crucians* seasonal history, 1946–1948. (Dashes show inadequate data.)



GRAPH II. *A. crucians* densities, 1946–1948 weekly average per station. (December and February less than 0.5.)

HIBERNATION

It is suggested that the winter behavior of *A. crucians* warrants use of the term hibernation. It must be sharply distinguished, however, from the hibernation of *A. quadrimaculatus* which occurs as inseminated females. In *A. crucians* the female seems always to develop eggs following a blood meal—there is no evidence of gono-

trophic dissociation in the fall. It is difficult to be quite certain of this since 90%, approximately, of November dissections have been found with relatively new blood meals. At any rate, the vast preponderance of females develop ova, not fat bodies. The large winter larval populations are the progeny of these November females. Furthermore, adults have never been found when pupae could not have been a fairly recent source, or when males were not also present if sought diligently enough. The earliest (February)[1] adults are invariably fresh, contrastingly-colored individuals. Our field evidence strongly indicates that A. *crucians* passes the winter period—December and January and sometimes until mid-February—almost entirely as larvae. In December none, and in January very few adults emerge. Thus, the aquatic stage of *A. crucians* in winter is greatly extended, probably often exceeding one hundred days. Larvae grow slowly and at different rates, especially in different ponds, and they seem to be more numerous and more active on mild, sunny days. The essence of hibernation of *A. crucians* in this area seems to be a dropping out of stages, other than larval, and a greatly delayed larval development.

SUMMARY

In the vicinity of Manning, South Carolina, *Anopheles crucians* survives the winter wholly in the aquatic stages, and undergoes retarded larval development. In contrast, hibernation by the inseminated female is typical for local *A. quadrimaculatus*, while *A. punctipennis* here breeds actively all winter.

REFERENCES

Barber, M. A., Komp, W. H. W., and Hayne, T. B., 1924. Some observations on the winter activities of Anopheles in southern United States. Public Health Rep.: 231.

Boyd, Mark F., and Weathersbee, A. A. 1929. Studies on the Bionomics of North American anophelines. V. Winter activities of anopheline imagines in coastal North Carolona (36° N. Lat.).
Am. Jl. Hyg. **9**(3): 682–694.

Hinman, E. H., and Hurlbut, H. S. 1940. A study of winter activities and hibernation of *Anopheles quadrimaculatus* in the Tennessee Valley.
Am. Jl. Trop. Med. **20**: 431.

[1] Unusually mild December and January weather in 1948–1949 so accelerated development of wintering larvae that consequential numbers of both sexes actually appeared on the wing in January, about three weeks earlier than usual in this area. January adults, observed further south by Barber, et al. (1924), obscured the characteristic differences in winter behavior of *A. crucians* from *A. quadrimaculatus* and *A. punctipennis*.